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The role of parent-offspring communication in resolving parent-offspring conflict in the burying beetle *Nicrophorus vespilloides*

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Lay summary

Parents and offspring do not always see eye to eye when it comes to the amount of parental care given and received during the growing period of the offspring. Offspring would benefit from receiving more care than the parents are willing to provide, thus leading into a conflict between the two. This conflict has often been thought to be alleviated through communication, where the offspring are capable of signalling their needs to the parent, which it can then use to assess whether the needs of the offspring match its own ability to provide more care without affecting its future ability to produce more offspring. Communication between parents and offspring mostly constitutes of the offspring begging for food from their parents via a variety of ways, from emitting odours that the parent can recognise, to using sounds or touching the parent. The burying beetle larvae beg for food from their parents by touching them with their feet, to which the parent responds by regurgitating food for them. The beetles live on small bird and mammal carcasses, which the larvae use as a food source, and where both parents provide elaborate parental care to them. I found that larvae are capable of telling apart different types of adults, including making a difference between other breeding beetles that they had not encountered before. Begging is also not dependent on the initial egg size of the larvae, suggesting that small and large larvae beg as much. Therefore begging is not an innate ability dependent on the initial quality of the offspring, but rather adjusted based on outside cues, such as parental state. I investigated whether begging would change parental response to begging, if the levels of begging were kept constant over the growing period of the larvae. The parents adjusted their responsiveness to the levels of begging they encountered, but not to fully respond to the need through the whole growing period. Finally, I tested whether this adjustment would cause impaired parental performance later in life, in terms of raising more broods, or their survival. I found that parents that increased their care in the first broods, laid fewer eggs, but survived just as well as control parents. In conclusion, I show that offspring can recognise breeding beetles from one another, and adjust their begging accordingly, but do not beg differently based on their own state. Offspring begging influences parents both in the short term, and also affects their future performance.

Abstract

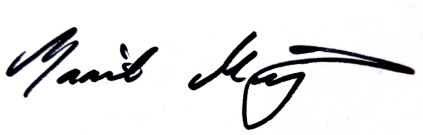
Parent-offspring communication is widely regarded as having evolved to provide the parent with honest information about the hunger state of its offspring, thus enabling it to mediate conflict over resource allocation between parents and offspring. The conflict is caused by the offspring benefitting from receiving more care than the parents are selected to provide due to the costliness of care. I studied the role of parent-offspring communication as a mediator for the conflict in the burying beetle *Nicrophorus vespilloides*. The burying beetle is an excellent study system for this question, as the larvae, that are raised on carcasses of small vertebrates and cared for by both the male and the female beetle, beg for food from their parents with highly distinguishable begging displays. First, I examined whether offspring adjusted their begging to different classes, or individual adult beetles. I found that while the larvae did not discriminate between male and female beetles, they adjusted their care to cues indicating individual recognition of adults. Second, I tested whether begging was based on offspring size at egg stage, and found no indication that offspring adjusted their begging to improve their innate quality. Third, I examined whether parental response to begging exhibits behavioural plasticity when the internal clock for the timing of reproduction for the parent, and the demand from the larvae do not meet. I found that the parents adjusted their care based on the amount of begging exhibited by the larvae. Fourth, I investigated whether parental adjustment of care based on offspring begging incurs a reproductive cost to them. I found that the females paid a cost in fecundity, but not in the number of dispersing larvae or their own survival. My original contribution to knowledge is therefore to show through these four studies, that offspring begging is adjusted based on parental cues, and can directly affect proximate parental behaviours, and also incurs a reproductive cost to their future reproductive success, thus providing more experimental evidence for the importance of parent-offspring communication, and its implications to the evolution of parental care.

Declaration

I declare that I have written this thesis under the guidance from my supervisor. I conducted all experimental work with help as stated below. All other work was my own.

Chapter 2: I conducted the majority of data collection, with contributions by Deborah Leigh and Daniel Collette. I performed all the analyses presented here, and wrote the subsequent manuscript and chapter in collaboration with my supervisor Per Smiseth. The original design of the experiment was established by Per Smiseth and Clare Andrews.

This work has not been submitted for any other degree or professional qualification.



Maarit Mäenpää

Acknowledgements

At the age of 10, I could think of nothing better than catching insects and watching them in their small jars, neatly arranged in rows at the corner of my parents' garden where I set up my first laboratory. I may not have fully understood what a biologist was at the time, but I was always determined to become one. The same enthusiasm for the living world that back then made me convince my family members to fill their pockets with caterpillars and lizards when mine were already full, later led me to my PhD.

There are two sometimes overlapping groups of people I owe my thanks to for their impact on the work presented here. First, there are the people who helped me with the science: I am grateful to my supervisor, Per Smiseth, and my current and former lab group members; Roni Mooney, Clare Andrews, Sarah Mattey, Charlotte Regan, Natalia Pilakouta, Lucy Ford, and Matthieu Paquet, as well as the other lab - Jacob Moorad, Ashleigh Whiffin, Edward Ivimey-Cook, and Ben Whittaker. Per - thank you for your support and encouragement over the years. There were definitely times when you believed in me more than I believed in myself. Roni - thank you for the friendly induction into the practical aspects of beetle-keeping. Sarah and Ashleigh - you made all that time spent in the windowless lab much nicer than it would have been otherwise. My thanks also go to Michael Dye, Peter Doe, and Peter Grantham, for all their practical help, as well as the good company. I also owe a thanks to the people who were happy to spent coffee breaks discussing statistics with me, and giving me second opinions. For this I owe my gratitude to many, but especially to my helpful statistically minded friends: Rebecca Callaby, Alyson Pavitt, Caroline Thomson and Becky Watson.

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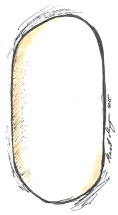
My greatest gratitude goes to my friends. By this I mean those of you whose name I mentioned, and those whose name I didn't, and those who were there for me from the beginning to the end, and to those who only dropped in briefly. I want to thank you for keeping me company, for distracting me, for giving me a place to stay when I needed one, for feeding me or the Beef, for laughing with me, for laughing at me, for sharing your worries with me and for listening to mine, for going on adventures with me, whether they be on tabletop or outside in the real world, for all the deep and meaningful conversations, and for all the nonsensical chatter, for the dancing, for the throwing of stones, and for the cake. Thank you for giving me a life to maintain beside the science. Research may be my passion, but, you, my friends, have given me memories worth more to me than any scientific endeavor I will ever embark upon.

Chapter 1

General introduction



1.1 Why study parent-offspring communication?

Parent-offspring communication, including offspring begging behaviour, has important implications for the resolution of parent-offspring conflict, although there is no consensus over the extent at which it operates, and how it has evolved (Chappell et al., 1995; Horn et al., 1995; Kilner and Johnstone, 1997; Kilner, 2001; Leech and Leonard, 1996; Rodríguez-Gironés et al., 2001; Soler et al., 2014; Wright and Leonard, 2002). Gathering further information about the nature of begging, including the mechanisms determining it, and its short- and long-term consequences to either the offspring or the parent, has important implications to our understanding of the evolution of parental care. Begging is usually thought of as a honest and reliable signal of offspring need (Godfray, 1995a,b), but the conspicuosness of begging may suggest it is also used as a means of manipulating the parent into allocating more resources than it would otherwise be willing to provide (Kilner, 2002). Some evidence suggests that offspring beg at different intensities based on their ability to recognise their parents (Bradbury and Vehrencamp, 2011), and there is also some indication of offspring learning to beg at different intensities based on parental behaviour (Kedar et al., 2000; Rodríguez-Gironés et al., 2002). However, the extent of recognition and discrimination that the offspring across different taxa exhibit, is still largely unknown.

Offspring can also beg based on different sources of need - they can either base their begging on their short-term needs, which can change rapidly (such as hunger), or of their long term needs that represent the total amount of investment needed through the juvenile period (such as condition) (Price et al., 1996). Many studies across a wide variety of taxa have given evidence that begging is adjusted based on the hunger levels of the offspring (Bell, 2008; Kilner and Johnstone, 1997; Leonard and Horn, 1996; Manser et al., 2008; Weary et al., 1996), but only a few studies have demonstrated changes in begging based on long-term need (Price et al., 1996). Because begging is also a powerful signal that can influence parental behaviour (e.g. Hinde et al., 2010; Meunier and Kölliker, 2012), it has the potential to affect parental traits not

only in the short-term but to also have carry-over effects to the later life of the parent. It is possible that caring for highly demanding offspring poses a reproductive cost on the caring parent, through lowering its chances of future reproduction or survival. My aim is to address these gaps in knowledge in order to gather more knowledge about the role of begging in resolving parent-offspring conflict, and through that, the evolution of parental care.

1.2 Parent-offspring communication

Parental care is defined as any behaviour that increases the survival and growth of the offspring, often at a cost to the survival or reproduction of the parent (Clutton-Brock, 1991; Royle et al., 2012). It can be directed towards different stages of offspring development, and varies in its form from being exclusively apparent either in the embryonic stage of development, or in the post-natal stage, and it can also be a combination of the two (Royle et al., 2012). In species where the parents and the offspring repeatedly interact with one another, most often when the parent repeatedly provisions food for the offspring, the amount of care given is affected by an information exchange between parents and the offspring via either the use of cues (i.e. traits evolved for other purposes, but which inadvertedly provide other information) or signals (i.e. traits evolved to produce the desired signal) (Maynard-Smith and Harper, 1995). A variety of offspring traits can be used to communicate with the parents: these traits can be subtle (e.g. chemical signals; Mas and Kölliker, 2008; Smiseth et al., 2010), or exuberant (eg. combination of offspring signals including behaviour and beak colour in altricial birds; Kilner and Johnstone, 1997). A large part of parent-offspring communication is comprised of solicitation signals expressed through elaborate offspring begging displays, that are used to extract resources from the parents (Kilner and Johnstone, 1997). These displays can be used to elicit a variety of commodities, such as parental warmth (Evans, 1994; Weary et al., 1996), or even defence (Cocroft, 1999), but mainly it is aimed at attaining food from the parents (Wright and Leonard, 2002).

Begging displays often encompass multiple traits of offspring morphology, physiology, and behaviour (as reviewed in Kilner and Johnstone, 1997; Mock and Parker, 1997; Royle et al., 2012; Wells, 2003; Wright and Leonard, 2002).

For example, nestlings of altricial birds use the colour of their gaping peaks (Pycraft, 1907), posture (Redondo and Castro, 1992), and vocalisation (Haskell, 1999) to attract the attention of their parents, and they also jostle one another to gain a better position than their siblings (Mcrae et al., 1993). Each element of the complex begging displays may have evolved due to their separate, discrete functions, and these multiple elements that comprise a signal may also have evolved to maintain its reliability (as reviewed in Kilner, 2002, and discussed further in section 1.3). Usually, the begging signals of dependent offspring increase in intensity as offspring need increases (Kilner and Johnstone, 1997), even though most evidence collected is based on short-term needs, such as hunger (Bell, 2008; Kilner and Johnstone, 1997; Leonard and Horn, 1996; Manser et al., 2008; Weary et al., 1996). Offspring of yellow-headed blackbirds also beg based on their long-term needs, as their condition affects their begging intensity when the short-term needs were controlled for (Price et al., 1996). Typically, changes in begging lead to corresponding changes in parental food provisioning rates, although parental response to begging also varies based on parental state (e.g. Thorogood et al., 2011). Individual parents can respond by ignoring the signal completely, or by increasing their food delivery rates in order to allocate more resources to the begging offspring (Royle et al., 2012; Wright and Leonard, 2002). Different parental responses can thus change the costs of begging and thus the marginal fitness benefits of the behaviour for the offspring, indicating that parental supply and offspring demand affect one another and therefore potentially coevolve (Grodzinski and Johnstone, 2012).

Aside from begging, parent-offspring communication also involves signals and cues that are transmitted from the parent to the offspring (Bradbury and Vehrencamp, 2011; Royle et al., 2012). The information conveyed from the parent to the offspring varies from cues that inform the offspring of parental identity (e.g. individual identity, sex, or breeding status), to directive signals that warn mobile broods against predators, maintain cohesion, or identify appropriate food items (Bradbury and Vehrencamp, 2011). In a few species, the parents also teach their offspring about food handling and environmental hazards (reviewed in Caro and Hauser, 1992), and offspring of a few species may also learn to alter their begging intensity based on the threshold of responsiveness in their prior experiences (e.g. Kedar et al., 2000; Rodríguez-Gironés et al., 2002). As parental care is costly, it is beneficial for the parent to recognise its own offspring, as it helps the parent to avoid bestowing

care to offspring that are not related to them (Bradbury and Vehrencamp, 2011; Royle et al., 2012). Individual recognition (Tibbetts and Dale, 2007) is normally favoured when the broods are mobile or raised in large communities, whereas shared family signals are favoured when broods are large, isolated, or raised in nests or burrows (Bradbury and Vehrencamp, 2011). In similar situations, it is beneficial to the offspring to recognise its own parent, as unfamiliar adults may pose a threat to the offspring, and costly begging without reward would also be a waste of resources (Wright and Leonard, 2002). While empirical evidence on offspring learning (e.g. Kedar et al., 2000; Rodríguez-Gironés et al., 2002) and recognition (Aubin and Jouventin, 1998; Lessells et al., 1995; McDonald et al., 2007) has started to accumulate in mainly avian species, further studies are still needed to adress the mechanisms of recognition, and the existence of learning in both avian and non-avian species.

1.3 Parent-offspring conflict

As both offspring begging and parental responsiveness to it are costly behaviours, for the two to have coevolved, both parents and the offspring must gain benefits from these behaviours. The benefits received by either party do not, however, always meet, as the parents and offspring disagree to a certain extent over the amount and duration of parental investment provided (Trivers, 1974). The result is a conflict between the parents and the offspring, where the offspring prefer receiving more parental investment than the parent is optimally selected to provide (Godfray, 1995b; Hamilton, 1964; Parker and Macnair, 1979; Trivers, 1974). Parent-offspring conflict can operate on different levels: Firstly, in intrabrood conflict, individual offspring within a brood aspire to receive more resources than the parent is willing to give (Macnair and Parkert, 1979). Secondly, a conflict between broods arises, when the offspring of one brood are effectively demanding resources that would benefit the parent more if allocated to future reproduction (Lessells and Parker, 1999; Parker and Macnair, 1979; Trivers, 1974). Offspring begging is strongly linked to parent-offspring conflict, as it is thought to play a key role as a mechanism for resolving parent-offspring conflict both within and between broods (Godfray, 1995b).

If offspring begging is used to manipulate the parent, in order to extract more

care than it is willing to provide, the offspring are effectively capable of lowering the parent's fitness when successful (Trivers, 1974). Manipulative offspring would thus exaggerate their begging displays beyond their actual needs (Trivers, 1974). While manipulation is beneficial from the offspring perspective, it poses a problem for the evolutionary stability of parent-offspring communication, as parents of manipulative offspring should be under selection to ignore these manipulative behaviours. Parent-offspring conflict is likely to be at its most severe when the costs of manipulation are low for the offspring, or if these costs fall on all members of the brood and not just the manipulator (Clutton-Brock, 1991). However, if the offspring are deceitful, the parent should eventually evolve mechanisms to prevent exploitation. A parent might, for example, escape manipulation by reducing its responsiveness to begging (Davies, 1976), showing aggression towards the offspring (Leonard et al., 1991), or even by abandoning the offspring (Reiter et al., 1978). Offspring, in turn, can affect the future clutch size and the likelihood and timing of future reproduction of the parents through begging (e.g. Hinde et al., 2010; Meunier and Kölliker, 2012), even without exaggerating their true needs. The potential influence that both parents and the offspring thus have on one another leads to the coevolution between parental supply and offspring demand, with the offspring's behaviour selecting for the amount of investment offered by the parent, and the parent's behaviour selecting for the intesity of offspring demand (Kölliker, 2003; Parker and Macnair, 1979; Royle et al., 2012). Therefore, understanding begging behaviour has important implications, not only to the resolution of parent-offspring conflict, but also to the evolution of parental care in itself.

1.3.1 *Theoretical models for parent-offspring conflict*

Godfray (1995a,b) proposed a model where begging accurately depicts offspring need, which would allow begging to evolve as an evolutionary stable strategy as long as the parents respond to it positively, and there is a cost to the behaviour that prevents cheating. According to these models based on the handicap principle, reliable begging signals advertising offspring need would ensure that food was given to the neediest offspring (Godfray, 1995b). Similarly, the offspring would be able to influence parental visitation rates through begging by conveying information of offspring condition to the parent (Godfray, 1991). According to another theoretical model, the offspring use begging in scramble

competition for food within the brood (Parker and Macnair, 1979; Parker et al., 2002b). The scramble competition models do not equate that begging has to be honest, as the intensity of begging indicates offspring quality (Parker and Macnair, 1979; Parker et al., 2002b). In scramble competition models, if the parents match offspring need exactly, the costs to begging dissipate, thus potentially lessening the need for honesty for begging in these situations (Parker and Macnair, 1979; Parker et al., 2002b). In this case, however, the offspring may influence parental food provisioning rates by manipulating the parent into providing more food, thereby preventing further wasteful solicitation (Eshel and Feldman, 1991; Johnstone, 1996; Parker and Macnair, 1979). These two models mark the opposite ends of a continuum from high (honest signaling) to low (scramble competition) parental power, and their prevalence depends on the breeding strategy and situation of the species in question (Royle et al., 2002).

While the theoretical models introduced above have indicated the need for the begging to be costly in order to maintain the honesty of the signal, many empirical studies have revealed apparently low signaling costs among relatives, raising the question of the ability of these models in explaining the evolution of begging behaviour (Andrews and Smiseth, 2013; Chappell et al., 1995; Horn et al., 1995; Kilner, 2001; Leech and Leonard, 1996; Rodríguez-Gironés et al., 2001; Soler et al., 2014). However, a multitude of case-specific theoretical models have shown situations where reliable signaling can still arise with low or even non-existent costs to begging, for example if costs of begging are dependent on the quality of the individual (Hurd, 1995), when cheating is detectable and punishable (Viljugrein, 1997), or when the pay-offs and signal costs of begging vary in a population (Maynard-Smith, 1994). More generally, costly begging and cost-free begging represent two different types of equilibria: one with distinct signals associated with distinct signalers, and one with pooled signals shared by multiple signalers (Bergstrom and Lachmann, 1998). An example of a distinct signal would be a mother bird that assesses the vocalisation volume of each of her offspring individually to determine the need for more resources, whereas a pooled signal would allow the mother to determine which of its offspring are begging, but using the total volume of the pooled as a signal of the hunger within the whole brood (Bergstrom and Lachmann, 1998). Similarly to cost-free models, other theoretical models (Payne and Rodriguez-Girones, 1998; Rodríguez-Gironés et al., 1996, 1998) have also questioned the evolutionary stability of handicap models, by pointing out that the signaling systems are also

stable, when no offspring beg at all. In fact, in computer simulations testing the spread of the signaling systems when the other equilibrium was taken into account, when a population started with no begging, it did not spread across the populations (Rodríguez-Gironés et al., 1998). Therefore for offspring begging to have evolved as a handicap signal, it needs to overcome a threshold of responsiveness from the parent, which is possible, if the offspring, for example, exploit sensory biases (Payne and Rodriguez-Girones, 1998; Rodríguez-Gironés et al., 1996, 1998). Cost-free signaling models and threshold models solve a problem posed by costly signaling models, where evolutionary innovations that make signaling cheaper would eventually make costly signaling unstable and thus unlikely to be maintained (Godfray, 1995a).

1.3.2 *Where are we now?*

Empirical evidence across taxa offers no ubiquitous support for one theoretical model for the resolution of parent-offspring conflict (Kilner and Johnstone, 1997; Wright and Leonard, 2002). While the honesty of the begging signals has been shown across many taxa, at least with regard to the short-term needs of the offspring (as reviewed in Kilner and Johnstone, 1997; Wright and Leonard, 2002), evidence is still lacking for whether offspring begging represents their long-term needs (but see Price et al., 1996). Manipulation of the parent by the offspring also exists in certain taxa, and at certain points of juvenile development (Riou et al., 2012). There are also no overall conclusions about the costliness of begging across all taxa (Andrews and Smiseth, 2013; Estramil et al., 2014; Kilner, 2001; Rodríguez-Gironés et al., 2001; Soler et al., 2014). These models are not, however, mutually exclusive, and can even be in operation simultaniously at different levels (Royle et al., 2002). Therefore, gathering more knowledge about begging behaviour, how it operates, and how it affects the parent, is still necessary for attaining a better understanding of the degree of which these different models can explain parent-offspring conflict, or the factors driving parental care. Here I will address these questions, with a particular emphasis on both the mechanistic and functional basis of begging behaviour, as well as its effects on the caring parent.

1.4 The study system: burying beetle, *Nicrophorus vespilloides*

The burying beetle, *Nicrophorus vespilloides*, is an ideal study system for investigating parent-offspring communication due to its short life cycle and elaborate parental care. Like other species in its genus (Silphidae: *Nicrophorus*), *N. vespilloides* uses carcasses of small vertebrates as a food resource for its partially dependent larvae (Eggert and Müller, 1997; Scott, 1998). Although the larvae are capable of feeding on their own from the carcass, which the parents have prepared (Eggert et al., 1998), they also beg for food from their parents by touching the adult with their feet, and the parents regurgitate pre-digested carrion to provision the young as a response (Smiseth and Moore, 2002; Smiseth et al., 2003). The behaviours of begging and provisioning, as well as other forms of parental care provided by the parents, have strong fitness consequences for all family members (Scott, 1998). For example, parental care increases offspring growth (Lock et al., 2004) and survival (Eggert et al., 1998). Both parents are involved in parental care, although the females usually stay with the brood until the larvae disperse from the carcass into the soil to find a place to pupate, but the males abandon the brood earlier (Scott, 1998). Parental care behaviours in this species are easy to observe in a laboratory setting, where the pedigree of the beetles and the stochastic effects of the environment can be controlled for, therefore excluding the noise caused by these effects from the experiments.

The breeding cycle of *N. vespilloides* starts when both male and female adults find a suitable carcass to breed on. There is both intraspecific and interspecific competition for carcasses, and large beetles are more successful at securing the carcasses (Otronen, 1988). The parents remove all fur or feathers of the carcass, roll it into a ball, and bury it in the soil (Eggert and Müller, 1997). The females then lay eggs in the soil close by during the first 2-3 days, while both parents continue to maintain and prepare the carcass, including breaking the skin, and thus creating a crater from which the larvae can later feed (Eggert and Müller, 1997; Scott, 1998). Once the eggs start hatching, the male often deserts the female in search of another mate (Scott, 1998; Scott and Gladstein, 1993). Larvae can therefore be raised by both parents, just one parent, or even no parents at all (Scott, 1998). The parents keep guarding and maintaining the carcass, as well as provisioning for the offspring, until the larvae reach the

appropriate size for dispersing from the carcass into the soil to find a place to pupate (Eggert and Müller, 1997). In the soil, the larvae wander for approximately 5-7 days, after which they pupate (Eggert and Müller, 1997). After approximately a week, the pupae eclose to adult beetles, which reach sexual maturity in about 10 days post eclosion (Eggert and Müller, 1997).

Due to its ideal nature as a study system, much is known about the reproductive behaviour of *N. vespilloides*. Providing care is costly for *N. vespilloides* parents. It reduces the female's future investment in reproductive attempts, including fecundity (Ward et al., 2009). Females also assess the carcass they use for reproduction, and invest more in broods raised on large carcasses (Creighton et al., 2009). Body size is an important determinant of fitness in this species: Female size affects the number and size of offspring that the female raises (Rauter et al., 2010). Small females produce larger broods with more body size variation, whereas large females produce smaller broods with larger larvae (Rauter et al., 2010). Larger females have also been reported to lay larger eggs, and to provide more care for their offspring (Steiger, 2013).

1.4.1 *Begging in* Nicrophorus vespilloides

Begging in the burying beetles reflects the hunger levels of the larvae, and is as such considered to be an honest signal of need (Smiseth and Moore, 2004a, 2007; Smiseth and Parker, 2008). Larval begging behaviour changes over time as the larvae become more proficient in self-feeding (Smiseth et al., 2003). The behaviour peaks at 24 hours after hatching, and starts declining after that, until approximately 72 hours after hatching, which marks the point of transitioning to nutritional independence (Smiseth et al., 2003). No energetic costs have been found for begging (Smiseth and Parker, 2008), but offspring that beg more are at a higher risk of being targets of filial cannibalism, which the parents use as a means for brood reduction, thus maintaining the honesty of the begging signal through punishment costs (Andrews and Smiseth, 2013). Larvae beg differently towards different classes of adult beetles, exhibiting recognition of the breeding status of the parent (Smiseth et al., 2010), but not between adults of different stages of the caring period (Leigh and Smiseth, 2012). It is not known, however, whether the burying beetle larvae exhibit individual recognition of their own parents. The parents respond to changes in the levels of begging by providing

more care (Rauter and Moore, 1999; Smiseth and Moore, 2002), but whether they continue responding to begging similarly throughout the juvenile period is not yet clear. Eggs of *N. vespilloides* hatch asynchronously, leading to assymmetries in the age and size of the siblings (Smiseth et al., 2006). Junior larvae beg more than the bigger senior larvae, but the senior larvae are more successful in gaining access to the parent (Andrews and Smiseth, 2013; Rauter and Moore, 1999; Smiseth and Moore, 2007). Differences in initial egg size, and thus potentially the initial competitive ability of the offspring through size differences, can possibly cause differences in the begging behaviour of the offspring. It is, however, currently unknown how initial egg size affects the begging behaviour of the larvae.

While begging appears to be an honest signal of need, the begging in burying beetles may not fully follow the honest signaling models. The presence of parents exacerbates sibling competition, as larvae in larger broods receive less care than larvae in smaller broods, supporting the models for scramble competition among siblings being a driving force behind parental resource allocation (Smiseth and Moore, 2007). The two models are not mutually exclusive (Parker et al., 2002b), and many species of animals follow a strategy that is somewhere between the two extremes of the different theoretical models that suggest either parental or offspring control over parental care (Royle et al., 2002). Indeed, in *N. vespilloides*, previous studies suggest that larvae have a high degree of control over the duration and magnitude of parental care provided in the species (Leigh and Smiseth, 2012; Smiseth et al., 2003). However, parents may control the within brood resource allocation (Andrews and Smiseth, 2013), and seem to have heightened control over the duration of care when the broods are asynchronous (Smiseth and Morgan, 2009). The parents stay with the brood, and continue to respond to begging even after the larvae reach nutritional independence, suggesting that offspring have behavioural control over the termination of care (Smiseth et al., 2003). However, evidence from experiments directly assessing these questions are still lacking, and examination of the role of begging is yet to be conducted. While parental care and larval begging has been studied widely in the burying beetle, its mechanistic basis, as well as its potential influence on parental care is still unknown. With the vast literature on the subjects regarding the parental traits associated with care, it is an ideal study system for investigating offspring begging.

1.5 Aims

I aim to investigate the mechanisms determining begging behaviour, how it operates, and its effects on the caring parent, both in short-term and in long-term. First, I examine the mechanistic basis of larval begging behaviour, and its influence over parental traits in *N. vespilloides*. Larval begging in this species is triggered by chemical cues from the parents (Smiseth et al., 2010). These cues are used to discriminate between breeding and non-breeding beetles (Smiseth et al., 2010), but not to discriminate between parents from different stages of juvenile development (Leigh and Smiseth, 2012). It is, however, not known whether prior exposure to chemical cues of parents of different sexes trigger begging at different levels. Here, I adress this question, and aim to investigate the mechanisms that trigger larval begging (Chapter 2). Larvae may adjust their begging to cues received from the parent, but begging in the burying beetles also reflects the hunger levels of the larvae, and is as such considered to be an honest signal of need (Smiseth and Moore, 2004a, 2007; Smiseth and Parker, 2008). Begging can, however, reflect either short-term needs (such as hunger) or long term needs (such as the relative contribution required to reach a target weight at independence) (Price et al., 1996). It is not known whether begging in the burying beetles reflects the long term needs of the larvae, and therefore I address the question by examining the relationship between initial egg volume and begging behaviour, as egg volume may potentially determine the long term needs of the larvae (Chapter 3).

After examining the mechanisms driving larval begging, I aim to test how begging behaviour can influence the parents. Both the parents and the offspring can bias the amount of parental investment towards their own optimum, but the extent of offspring influence is still largely unknown (reviewed in Kilner and Johnstone, 1997). First, I investigate whether different levels of larval begging over an extended period of time during juvenile development induce changes in the levels of care in response to the changes in larval demand (Chapter 4). If offspring are capable of influencing the amount of care given during the first reproductive event, it is also possible that this increase in parental investment induces reproductive costs to the parent in terms of their future reproductive success or survival (Williams, 1966). I address this question by monitoring the second reproductive event and mortality of female beetles, whose first reproductive event was manipulated to have increased or decreased levels of

larval demand (Chapter 5). Together these experiments uncover both the mechanistic basis of how the larvae adjust their begging based on cues from the parent, and also how the parent adjusts its care based on the begging exhibited by the larvae. Understanding these interactions can thus also give us more insights into the role of begging in resolving parent-offspring conflict, as well as in the evolution of parental care.

Chapter 2

Burying beetle larvae discriminate between individual parents and between some classes of adults

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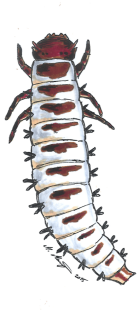

Abstract

Offspring begging can be triggered by a variety of acoustic, visual or chemical cues from the parents. In many birds, nestlings use information derived from these cues to discriminate between individual parents or different classes of adults. Although begging occurs in some insects, very little is known about discrimination between adults by insect larvae. Here, I examine whether begging larvae in the burying beetle *Nicrophorus vespilloides* can discriminate between individual parents or different classes of adults. I found that larvae showed no discrimination between male and female beetles, but that they begged more towards breeding beetles than towards non-breeding ones. These results were robust regardless of whether larvae had been reared in presence or absence of adult beetles, thus suggesting that larval discrimination is based on an innate template that requires no prior exposure to adult beetles. I also found that larvae begged more towards unfamiliar beetles than towards familiar ones, suggesting that they can learn to discriminate between individual parents based on cues about familiarity. I conclude that insect larvae may benefit from discriminating between different classes of adult beetles, as it allows them to lower the costs associated with begging in response to irrelevant environmental cues (costly in terms of wasted effort) and with not begging in response to the presence of caring parents (costly in terms of lost feeding opportunities).

2.1 Introduction

Offspring of many birds and mammals, as well as some insects, beg for food from their parents (Kilner and Johnstone, 1997). In many birds, begging is triggered by acoustic or visual cues from the parents, such as the feeding calls announcing the parent's arrival in many songbirds (e.g. Leonard and Horn, 2001; Madden et al., 2005) or the red patch on the parent's bill in herring gulls *Larus argentatus* (ten Cate et al., 2009; Tinbergen, 1948). This phenomenon is thought to reflect selection on offspring to reduce the costs associated with the

failure to start begging as the parent arrives at the nest (Budden and Wright, 2001; Leonard and Horn, 2001), and mistaken responses to irrelevant environmental noises (Leonard et al., 1997). In many birds, nestlings use information derived from these parental cues to discriminate between individual parents or different classes of adults (Lessells et al., 1995). For example, in king penguins *Aptenodytes patagonicus*, parents produce individually distinctive feeding calls, which chicks use to discriminate their parents from other adults in the breeding colony (Aubin and Jouventin, 1998). Although begging also occurs in some insects, there is little information on the offspring's ability to discriminate between individual parents or different classes of adults.

The burying beetle *Nicrophorus vespilloides* is an excellent system for studying begging (Smiseth and Moore, 2002, 2004b, 2007; Smiseth et al., 2003, 2007b, 2010). Like all members of its genus, this species breeds on carcasses of small vertebrates (Scott, 1998). Once a suitable carcass has been located, a male and a female normally cooperate by burying it underground, rolling it into a ball and removing fur or feathers (Scott, 1998). One or both parents provide care for the larvae by cleaning the carcass of microbial growth, defending the brood against predators and conspecific intruders, and provisioning regurgitated carrion to the larvae (Scott, 1998). Larvae beg for food from the parents by raising their heads and touching the parent (Smiseth et al., 2003). As predicted by theoretical models of begging as an honest signal (Godfray, 1991), begging reflects larval hunger levels (Smiseth and Moore, 2004b), parents adjust their food provisioning in response to begging (Smiseth and Moore, 2002), and begging is costly to the larvae (Andrews and Smiseth, 2013).

Previous work shows that larval begging in *N. vespilloides* is triggered by chemical cues from the parents and that larvae use these cues to discriminate between breeding and non-breeding females (Smiseth et al., 2010), but not between parents in different stages of breeding (Leigh and Smiseth, 2012). There is good evidence that breeding and nonbreeding beetles differ with respect to surface chemicals (cuticular hydrocarbons and methyl geranate), and adult females discriminate between their male breeding partner and non-breeding intruders based on differences in their surface chemical profiles (Haberer et al., 2010; Müller et al., 2003; Steiger et al., 2007). There is also evidence that male and female beetles differ with respect to surface chemicals (Haberer et al., 2010; Steiger et al., 2009), and adult beetles learn to recognise

individual differences in chemical cues (Steiger et al., 2008). In this study, I examine whether larvae can discriminate between different classes of adults based on their sex and breeding status. I also examine the role of learning by testing whether the larvae's ability to discriminate between specific parental cues requires prior exposure to breeding adults. Finally, I examine whether larvae can learn to recognise individual differences in parental cues.

2.2 Materials and methods

2.2.1 *Origin and husbandry of the beetles*

The beetles used in the experiments originated from an outbred laboratory population maintained at The University of Edinburgh, UK. The population descended from beetles caught in Corstorphine Hill and Craiglockhart Hill (Edinburgh, UK), and Kennall Vale (Cornwall, UK). All beetles were housed individually in transparent plastic containers (12 x 8 x 2 cm) filled with moist soil, and kept under constant light at 20°C. Non-breeding beetles were fed small pieces of organic beef twice a week. For breeding, pairs of non-sibling males and females were selected randomly and placed in a plastic container (17 x 12 x 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (10-20 g; supplied by Livefoods Direct, Sheffield, UK).

2.2.2 *General experimental procedures*

Across all experiments, I adopted the general protocol for recording larval begging in standardised broods comprised of 10 same-aged larvae presented with a standardised stimulus in the form of a dead adult beetle from a specific treatment group (Smiseth and Parker, 2008; Smiseth et al., 2010). This protocol provides an experimental procedure for excluding confounding effects due to variation in the size and age-composition of the brood (Smiseth et al., 2003, 2007b; **?**) or the behaviour of adults (Smiseth et al., 2010). To generate experimental broods, we moved the breeding pair and the carcass to a fresh container 65 h after pairing, thereby leaving the eggs to hatch in the original

container. I checked the original container multiple times each day for the presence of newly hatched larvae, which were used to generate experimental broods that always comprised of mixed-maternity larvae (for further details on the protocol and rationale for use of mixed-maternity broods, see Smiseth et al., 2010). I allocated experimental broods randomly to foster parents, only using beetles whose own eggs had started hatching to avoid filial cannibalism (Bartlett, 1987).

Larval begging was recorded 24 h (20 min) after the experimental broods had been generated to coincide with the stage in larval development when begging peaks (Smiseth et al., 2003). Thirty minutes before I started recording larval begging, I removed the adult beetle to be used as a stimulus (see Experimental Design for further details). I then killed the beetle by placing it in a -20 °C freezer for 20 min and left it to thaw for another 5 min before pinning it within a small plastic container (12 x 8 x 2 cm) lined with a moist paper towel (see Smiseth et al., 2010). The beetle was pinned in a position mimicking that of a parent regurgitating food. Once the beetle had been pinned, I removed the larvae and placed them next to the pinned beetle. I waited 5 min before starting the observations to give the larvae time to settle. I recorded larval begging using instantaneous recording every 1 min during a 30-min observation period according to established protocols (Smiseth and Moore, 2002). The average time spent begging by each larva in the brood, B, was calculated as $B = (b/L)/30$, where b is the total number of begging events during the 30-min observation period and L is the mean number of larvae near the adult during each scan (i.e. within 0.8 cm diameter from the pin). The total sample size across all three experiments amounted to 132 broods.

2.2.3 *Experimental design*

Experiment 1: In this experiment, I tested whether larvae that had been reared in the presence of both a male and a female beetle discriminated between the two sexes. I left both a male and a female beetle with the brood until I conducted the behavioural observations to ensure that the larvae had been exposed to the chemical profiles of both parents. Males often desert the brood during the first 24 h after hatching (Smiseth et al., 2005), and it is currently unclear whether the chemical profile of deserting males resembles that of caring

males. I therefore recorded all instances of male desertion, by checking twice whether the male (and female) was present on the carcass or the surrounding crypt before conducting the behavioural observations. I did the first check 30 min before the removal of the target parent and the second one immediately prior to it. If the male was absent from the carcass during both checks, I scored him as having deserted the brood. In summary, in this experiment, the larvae were presented with a standardised stimulus in the form of a dead adult from one of the following three categories: a caring female (n = 10), a caring male (n = 10) or a deserting male (n = 10). I presented all larvae with one of the adults that previously had provided care for them (hereafter referred to as a caring parent) to exclude any potential confounding effects due to the familiarity of the adult.

Experiment 2: This experiment was designed to test whether larvae that had been reared by a single male or female beetle discriminated between adults based on their breeding status or familiarity. I also tested whether any discrimination based on cues about the breeding status and familiarity was conditional on the adult's sex. I always removed one member of the breeding pair at the time I generated the experimental broods, thereby leaving the remaining beetle to provide care on its own during the first 24 h after hatching. I then presented the larvae with an adult beetle of the same sex as the beetle that previously had cared for them. I used a single-parent design because, when both parents care jointly, females tend to spend more time interacting with the larvae than males. In contrast, there is no difference in the amount of time that single males and single females spend interacting with the larvae (Smiseth et al., 2005). Thus, this design allowed us to exclude any potential confounding effects that may arise in Experiment 1 due to females interacting more with the larvae.

Previous work shows that larvae respond to cues about the breeding status of females (Smiseth et al., 2010), but there is no information on larval responses to cues from males. Thus, we tested whether larval discrimination between breeding and non-breeding adults was conditional on the adult's sex. To this end, I presented the larvae with either a breeding or non-breeding adult. There were no instances of desertion in this experiment, and all breeding beetles were caring for the larvae. Given that non-breeding adults inevitably will be unfamiliar to the larvae, I presented the larvae with an unfamiliar breeding adult as a control. Finally, this experiment also allowed me to examine whether

larvae can learn to recognise individual differences in chemical cues, as previously reported for adults (Steiger et al., 2008). If so, I expected larvae to discriminate among familiar and unfamiliar breeding adults. Thus, I presented some larvae with the adult that had previously provided care for them (hereafter referred to as a familiar breeding parent) and some larvae with an adult that had previously cared for a different brood (hereafter referred to as an unfamiliar breeding parent). In summary, the larvae used in this experiment were presented with a dead male or female beetle from one of the following treatments: a familiar breeding beetle (n = 11 and n = 10 for females and males, respectively), an unfamiliar breeding beetle (n = 10 and n = 10 for females and males, respectively) or an unfamiliar non-breeding beetle (n = 9 and n = 10 for females and males, respectively).

Experiment 3: In this experiment, I tested whether the larvae's ability to discriminate between adults based on cues about breeding status and sex required that the larvae had previously interacted with adult beetles. In *N. vespilloides*, larvae obtain some food by self-feeding, and they survive well without post-hatching parental care (Eggert et al., 1998; Smiseth et al., 2003). Thus, I reared larvae in isolation from caring parents during the first 24 h after hatching by removing both parents at the time I generated the experimental broods. I then examined whether these larvae discriminated between adults based on breeding status and sex by presenting them with a dead male or female from one of the following two treatments: a breeding beetle (n = 11 and n = 8 for females and males, respectively) or a nonbreeding beetle (n = 10 and n = 13 for females and males, respectively).

2.2.4 *Statistical analysis*

All analyses were conducted using R version 3.0.2 (R Core Team, 2013). I used a logit transformation with 0.001 as the constant ε to reduce heteroscedasticity and normalise the error structure of the proportional data on larval begging (Warton and Hui, 2011). The data were collected in multiple experimental blocks conducted at different times over 3 years. I used linear mixed-effects models (lme, package nlme, Pinheiro et al., 2014) to analyse data on all experiments, with block as a random effect. For Experiment 1, I used treatment (caring female, caring male, deserting male) as a fixed factor. This analysis

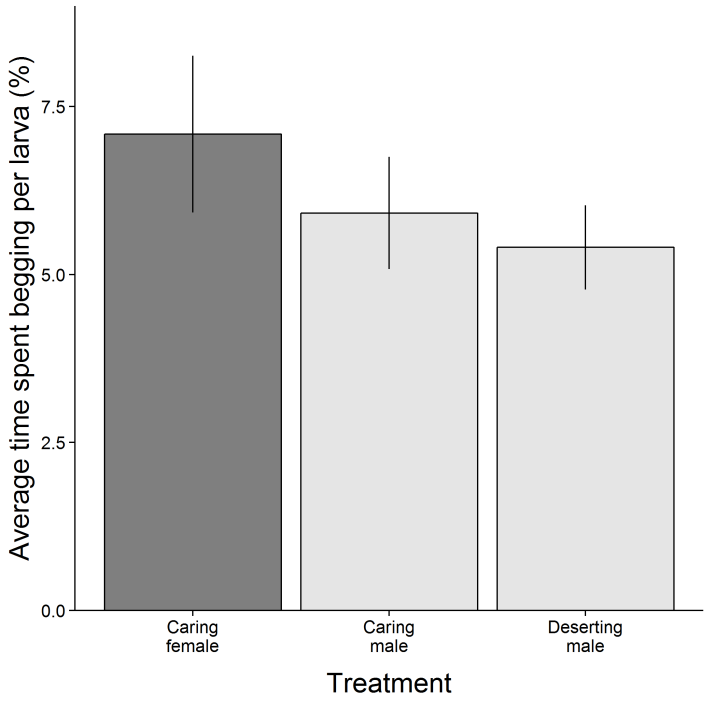

Figure 2.1: Mean (+/- SE) time spent begging by individual larvae (%) towards a dead parent. In this experiment, both parents had reared the larvae. There were no significant differences in the amount of time spent begging towards different classes of adults ($p < 0.05$).

allowed me to test for a difference in larval begging towards male and female beetles and towards caring and deserting males. For Experiment 2, I used treatment (the breeding status and familiarity of the adults, that is familiar breeding beetles, unfamiliar breeding beetles, unfamiliar non-breeding beetles) and sex of the adult (females, males) as fixed factors. This analysis allowed me to test for a difference in larval begging towards male and female beetles, towards familiar and unfamiliar breeding adults and towards unfamiliar breeding and non-breeding adults. For Experiment 3, I used breeding status (breeding beetles, non-breeding beetles) and sex of the adult (females, males) as fixed factors. This analysis allowed us to test for a difference in larval begging towards breeding and non-breeding adults. Contrasts comparing the different levels of significant main effects were computed to identify differences between groups for treatments that had more than two levels.

2.3 Results

Experiment 1: There were no significant differences in the amount of time the larvae spent begging towards caring females, caring males or deserting males

(lme, $F_{2,25}$=0.193, P=0.826). Thus, larvae that had been reared by both parents did not discriminate between caring parents based on their sex or between caring males and males that had deserted the brood (Figure 2.1).

Experiment 2: Larvae spent a similar amount of time begging towards females and males when they had been reared by either a single female or a single male beetle (lme, $F_{1,52}$=0.114, P=0.737). Thus, as in Experiment 1, larvae did not discriminate between adults based on their sex (Figure 2.2). However, there was a highly significant effect of treatment (i.e. the breeding status and familiarity of adults) on the amount of time that the larvae spent begging (lme, $F_{2,52}$=42.6, P <0.001). Post hoc contrasts show that the larvae spent significantly more time begging towards unfamiliar breeding beetles than towards familiar ones (z_{54}=6.65, P < 0.001, Figure 2.2). In addition, larvae spent significantly more

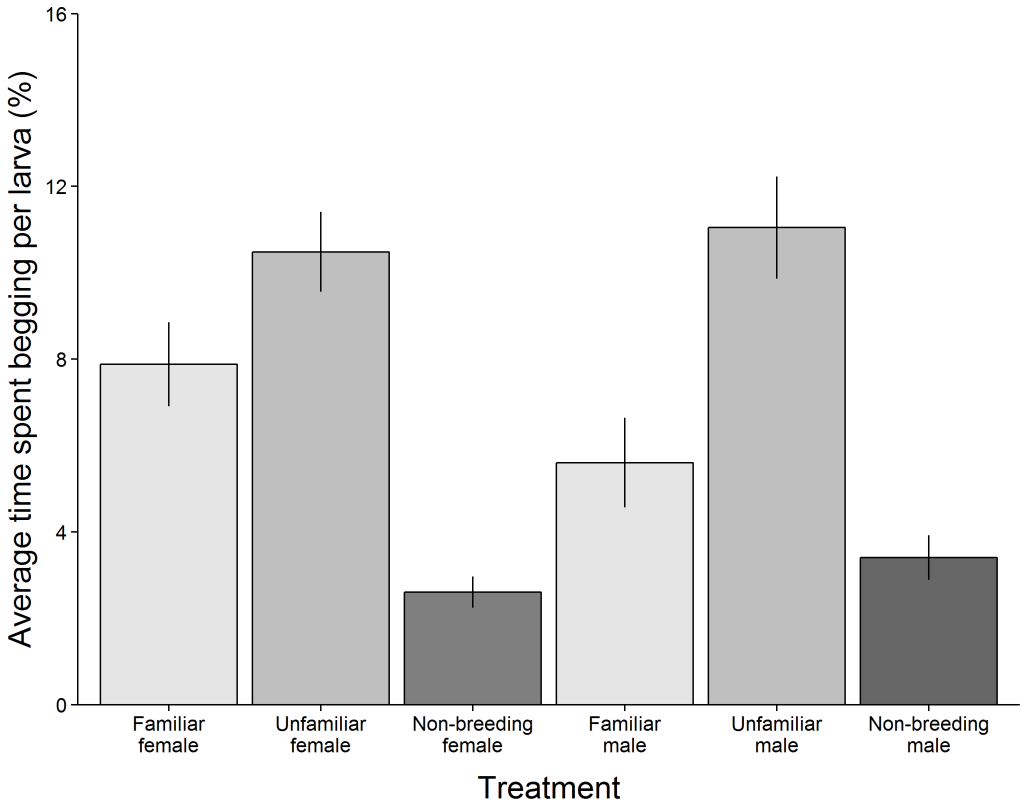

Figure 2.2: Mean (+/- SE) time spent begging by individual larvae (%) towards a dead parent. In this experiment, either a single male or a single female had reared the larvae. There were no significant differences in the amount of time the larvae spent begging towards a male or a female beetle (p <0.05), but the differences across all treatment types (familiar, unfamiliar and non-breeding) were statistically significant (p <0.05).

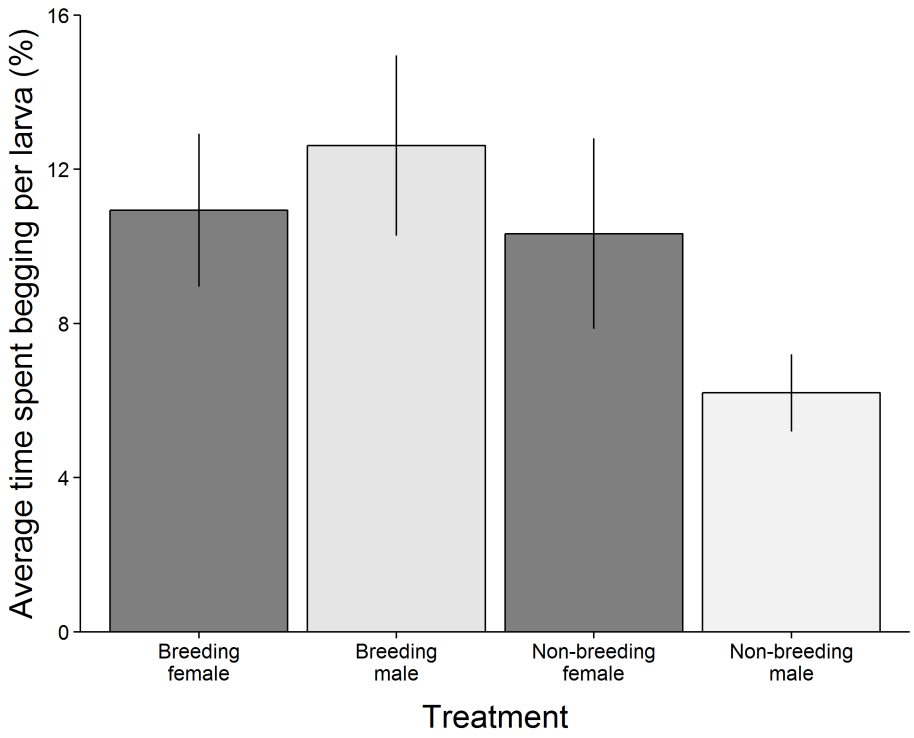

Figure 2.3: Mean (+/- SE) time spent begging by individual larvae (%) towards a dead parent. In this experiment, the larvae had been reared in isolation from caring parents. There were no significant differences in the amount of time spent begging towards a male or a female beetle ($p<0.05$), but the differences in begging towards breeding and nonbreeding adult beetles were statistically significant ($p<0.05$).

time begging towards unfamiliar breeding beetles than towards unfamiliar nonbreeding beetles ($z_{54}=6.08$, $P<0.001$, Figure 2.2). There was no significant effect of the interaction between treatment and sex on larval begging (lme, $F_{2,52}=2.21$, $P=0.137$).

Experiment 3: There was no significant difference in the amount of time spent begging towards females and males when larvae had been reared in isolation from any contact with adult beetles (lme, $F_{1,36}=1.58$, $P=0.217$). Thus, as in the previous two experiments, larvae did not discriminate between adults based on cues about their sex (Figure 2.3). As in Experiment 2, there was a significant difference in the amount of time spent begging towards breeding and non-breeding beetles (lme, $F_{1,36}=4.17$, $P=0.049$). Although visual inspection of the data seems to indicate a differential response to the treatments between males and females (Figure 2.3), there was no significant effect of interaction term between treatment and sex (lme, $F_{1,36}=2.85$, $p=0.100$).

2.4 Discussion

Here, I provide evidence that burying beetle larvae discriminate between certain classes of adults and between individual parents. I found that larvae did not discriminate between male and female beetles regardless of whether larvae had previously interacted with male and female beetles. There are three potential explanations for why larvae did not discriminate between males and females: (1) there are no cues available to the larvae about the adults' sex, (2) there are such cues but larvae cannot detect them, and (3) larvae can detect the cues but it is beneficial to ignore them. Previous work allows me to exclude the first explanation as it shows that males and females have different surface chemicals (Haberer et al., 2010; Steiger et al., 2009). It is harder to differentiate between the remaining two explanations. There is good evidence that adult females can detect cues about sex given that they are more aggressive towards dead females than towards dead males (Steiger et al., 2009). However, this does not necessarily mean that larvae can detect the same cues, as they may not have the same sensory and cognitive mechanisms for detecting and processing chemical cues as adults. However, even if larvae could detect chemical cues about the adult's sex, it may be detrimental to respond to them. The reason for this is that, even though females spend more time provisioning food to the larvae overall (Eggert et al., 1998; Smiseth and Moore, 2002), males and females are equally likely to provision the larvae when in close proximity to them (Smiseth and Moore, 2004b). Thus, any reduction in begging towards males is likely to incur a cost in terms of lost feeding opportunities. Based on available evidence, I propose that it would be beneficial for larvae to ignore cues about the parents' sex even if they could detect such cues. My finding on burying beetles is similar to results from a study on birds, showing that nestlings of European bee-eaters *Merops apiaster* do not discriminate between male and female parents (Lessells et al., 1995).

Larvae begged more towards breeding beetles than towards non-breeding ones regardless of whether they had been reared in the presence or absence of adult beetles. This is consistent with a previous study showing that larvae spend more time begging towards breeding females than towards non-breeding ones (Smiseth et al., 2010). My results extend on this work by showing that larval discrimination between adults based on breeding status is independent of the adult's sex and not conditional on prior experiences with adult beetles. There is

good evidence that breeding and non-breeding beetles differ with respect to surface chemicals, and adult females are known to use information from these cues to discriminate between their breeding partner and non-breeding intruders (Haberer et al., 2010; Müller et al., 2003; Steiger et al., 2007). It may be beneficial for larvae to respond to chemical cues about the breeding status of adults, because it would provide them with a mechanism with which larvae can reduce the costs associated with discriminating between the parent and irrelevant environmental cues in underground darkness (Smiseth et al., 2010).

The finding that larvae discriminated between breeding and non-breeding adults even when they had been reared in isolation from adult beetles suggests that larval discrimination between parental cues is based on an innate template that requires no prior exposure to adult beetles. Nevertheless, visual inspection of the data suggests that this effect was pronounced when larvae were presented with males but not when larvae were presented with females. Thus, larval discrimination appears to be weaker and less consistent when the larvae were reared in isolation from adults (Experiment 3, Figure 2.3) than when the larvae were reared by a single parent (Experiment 2, Figure 2.2). I argue that it would be premature to rule out the possibility that learning might play a role in moderating the larvae's responses towards chemical cues towards parents. Indeed, previous work shows that larvae adjust their begging behaviour in response to the number of competing larvae in brood (Smiseth et al., 2007b) and their own competitive rank relative to that of their siblings (**?**), suggesting that larvae moderate their begging behaviour to the competitive environment in which they find themselves possibly through learning. Furthermore, studies on birds provide good evidence that learning plays an important role in moderating the nestling's begging behaviour (Kedar et al., 2000; Lotem and Biran-Yoeli, 2014). Thus, there is a need for further experiments that address the potential role of learning as a mechanism for moderating larval begging behaviour.

Finally, I found that larvae begged more towards unfamiliar beetles than towards familiar ones, suggesting that they can discriminate between individual adult beetles. My results derive from an experimental design that excluded confounding factors that otherwise covary with familiarity. In natural broods, familiarity covaries with kinship because the larvae tend to be reared by their biological parents (Müller and Eggert, 1989). I can exclude effects of kinship on my results because all experimental broods in this study were reared by foster

parents. In natural broods, familiarity also covaries with breeding status because any familiar beetle inevitably will be a breeding adult, while any unfamiliar beetle is likely to be a nonbreeding intruder (Bartlett, 1987; Müller and Eggert, 1990). I can also exclude effects due to breeding status because the familiar and unfamiliar adults in my experimental design always had the same breeding status. Surprisingly, I found that larvae spent more time begging towards unfamiliar adults than towards familiar adults, a pattern that was consistent across both sexes. This finding contrasts with a recent study on jackdaws *Corvus monedula*, showing that older nestlings do not discriminate between the calls of their parents and other conspecifics, although they discriminated between conspecific calls and the calls of other corvid species (Zandberg et al., 2014). It is difficult to come up with an adaptive explanation for why larvae should beg more towards unfamiliar beetles. Potentially, this unexpected finding may reflect the outcome of the underlying sensory or cognitive mechanism that controls begging, which caused the larvae to respond in a non-adaptive way in a novel experimental setting (Fawcett et al., 2012). Larvae do not normally encounter unfamiliar non-breeding adults, and if they are neophilic, they may increase their begging when presented with a novel stimulus. Currently, it is unknown whether burying beetle larvae are neophilic or not, and further work is needed to establish what determines their response to novel cues.

My results support the suggestion that larval discrimination plays an important role as a mechanism for reducing the costs of begging. Previous work suggests that nestling birds are under selection to reduce the costs associated with the failure to start begging as the parent arrives at the nest (Budden and Wright, 2001; Leonard and Horn, 2001), and mistaken responses to noise in the environment (Leonard et al., 1997). My results suggest that burying beetle larvae are under similar selection pressures and that larval discrimination between adults serves as an adaptive mechanism that allows the larvae to lower the costs associated with begging towards irrelevant environmental cues (which is costly in terms of wasted effort) and with not begging in response to the presence of caring parents (which is costly in terms of lost feeding opportunities).

Chapter 3

Egg size is positively associated with offspring quality, but not begging behaviour

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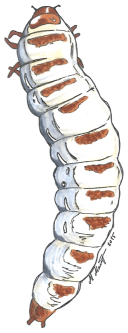


Abstract

Egg size and parental care are important parental traits determining offspring fitness, but there is no consensus about the potential mechanisms through which the two have coevolved. Comparative evidence suggests that species with elaborate forms of post-hatching care produce smaller eggs than species with lesser forms of post-hatching care, and post-hatching parental care has also been shown to mask the effects of initial egg size. A potential mechanism for the masking effect of care could be that parents provide post-hatching care at different levels to offspring originating from different sized eggs, using cues from offspring begging. The potential association between egg size and offspring begging behaviour could also propose a mechanism for other previously discovered associations between egg size and parental care. For example, it could explain why large females have been found to produce both larger eggs and provide more post-hatching care. Here I propose that this association could potentially exist in either negative form, to facilitate compensatory growth potentially explaining the masking effects of parental care, or in positive form as an additional offspring quality trait correlated with fitness benefits due to size. In this study, I directly studied the association between egg size and begging behaviour in the burying beetle *Nicrophorus vespilloides*. I also examined the effects of egg size on offspring growth and development. I found no association between egg size and begging, indicating that begging is not a mechanism for either of the proposed scenarios. Egg size, and the subsequent offspring size, did, however, correlate positively with other offspring fitness traits (development time, size, and survival), leading to potential benefits for producing larger eggs even for species with elaborate post-hatching care.

3.1 Introduction

The coevolution of parental care and egg size, both of which are important parental traits determining offspring's fitness, is a subject of debate in evolutionary biology (Gilbert and Manica, 2010; Nussbaum, 1985, 1987;

Nussbaum and Schultz, 1989; Sargent et al., 1987; Shine, 1978, 1989). Evidence from comparative studies suggests that species which provide care for their offspring at the egg stage lay larger eggs than species that direct their care towards later stages in offspring development or provide no care at all (Gross and Sargent, 1985; Kolm and Ahnesjö, 2005; Nussbaum, 1985, 1987; Sargent et al., 1987; Shine, 1978, 1989). There is, however, no clear consensus for the evolutionary explanation behind the association between parental care and egg size. According to the safe harbour hypothesis, the parents reduce egg mortality rates in relation to juvenile mortality rates by providing care for the eggs, causing selection to favour large eggs and to minimise the time spent in the hazardous juvenile phase (Shine, 1978). Alternatively, if egg mortality increases with egg size, and can only be counteracted through increased parental care, selection would favour parental care at the egg phase (Nussbaum, 1985, 1987). For example, in aquatic organisms large eggs require more oxygenation, and thus receive more care than small eggs (Nussbaum, 1987; Nussbaum and Schultz, 1989). Egg size and parental care may also have coevolved as a part of a life history strategy favoured by selection as an adaptation to the amount of food available for the offspring, and thus the harshness of the environment (Itô and Iwasa, 1981). Recent empirical evidence has suggested that the relationship between parental care and egg size is not as straightforward as the present models suggest, as post-hatching parental care can also mask the initial effects of egg size on offspring development (Monteith et al., 2012). This finding presents important implications for the theoretical work, as it underlines the differences between pre- and post-hatching parental care, and implies that selection should work to drive the egg sizes of species providing pre- or post-hatching care further apart. It is important to consider the relationship between egg size and parental care for both types of care, as many species including birds and mammals (through pre-natal resource allocation with the placenta in place of eggs) frequently exhibit both (Royle et al., 2012).

Given that post-hatching parental care may mask the effects of initial egg size (Monteith et al., 2012), it would be logical for the species with elaborate post-hatching care to produce smaller eggs than the species offering mostly pre-hatching care. Some comparative studies offer support for the hypothesis: In birds altricial species with elaborate forms of post-hatching care have smaller eggs than precocial species, whose offspring are highly independent straight after hatching (Wesolowski, 1994; Williams, 1994). In insects, species that

provide any levels of care to their offspring produce eggs that are no different in size to those of species that provide no care at all (Gilbert and Manica, 2010). These widespread associations between small egg sizes and post-hatching care between species indicate that the effects of post-hatching care are potentially strong enough to swamp the smaller effects of egg size across a wide range of taxa. However, an alternative explanation to the results found by Monteith et al. (2012), is that the females adjust their effort to compensate for the initial effects of egg size through care, thus providing more care for the offspring originating from smaller eggs. Parents often adjust the amount of care given based on offspring cues as well as cues of their own state (Creighton et al., 2009; Kight, 1997; Thorogood et al., 2011, Chapter 2). The mechanism for parental adjustment of care is often parental food provisioning, through which the parent can match the amount of care provided with the need of the offspring, who in many cases express it through elaborate begging displays (Kilner and Johnstone, 1997). Offspring begging may either reflect their short term need (i.e. hunger), or their long term need (i.e. condition relative to either the amount of resources required for reaching a target body mass at independence, or other offspring in the brood) (Price et al., 1996). However, whether initial egg size causes differences in offspring long-term needs, that are then reflected on their begging behaviour, is still unknown.

In a meta-analysis on birds, Krist (2011) found that egg size correlates widely with a variety of offspring fitness traits, implying that there is a positive correlation between offspring quality and egg size. Nevertheless, larger eggs do not necessarily equate to better quality offspring: For example, Jacobs and Sherrard (2010) found no positive effects of initial size on growth rate or survival in ascidians, and found no evidence for interspecific competitive advantage due to initial size either. Similarly, Régnier et al. (2013) found that small offspring survived at higher rates than large offspring in brown trout (*Salmo trutta*). Furthermore, egg size and composition is largely due to the quality of the mother that lays it, mostly resulting in large females, or those in good condition, laying larger eggs than their small conspecifics in poorer condition (Parker and Begon, 1986). Nevertheless, in groups of butterflies and some passerines, female condition has also been reported to correlate negatively with egg size (Christians, 2002; Fox and Czesak, 2000). Female condition can also be affected by different traits linked with parental state, and not all of them necessarily have the same effects: Kojima (2015) found that in giant rhicoceros

beetles (*Trypoxylus dichotomus*) small eggs produced by old females reached a large size at pupation due to faster development and compensatory growth, but small eggs laid by small females remained small through their development, and showed no signs of compensatory growth. Parental size has been found to correlate with a variety of parental traits affecting offspring fitness: Steiger (2013) found that large female burying beetles (*Nicrophorus vespilloides*) layed both larger eggs, and provided more post-hatching parental care than their small conspecifics, indicating that bigger mothers were of a higher quality than smaller ones. Steiger (2013) did not, however, measure offspring begging, and thus an alternative explanation to the positive correlation between egg size and parental care may be that offspring from larger eggs begged more, thus extracting more care from their parents than offspring of smaller eggs do. Therefore, a relationship between egg size and begging behaviour could potentially exist in either negative form, to facilitate compensatory growth potentially explaining the masking effects of parental care, or in positive form as an additional offspring quality trait correlated with fitness benefits due to size.

The burying beetle, *Nicrophorus vespilloides*, is an ideal study system for investigating the association between egg size and parental care, as it provides elaborate post-hatching parental care for its offspring, which it raises on the carcasses of small vertebrates (Scott, 1998). Parental care is not obligatory for the survival of the young, but is has been shown to greatly improve the fitness of the larvae (Smiseth et al., 2007a). Furthermore, the burying beetle is the species where both parental care masking the effects of initial egg size (Monteith et al., 2012), and a parental size effect correlating both with egg size and post-hatching care (Steiger, 2013) were reported. In this study, I directly investigate the association between egg size and offspring begging behaviour, as well as the effects of egg size on other offspring traits, including their survival. Data on these relationships is still largely lacking (Krist, 2011), and here I intend to adress that gap in knowledge.

3.2 Materials and methods

3.2.1 *Experimental design*

The general outline of the experimental set-up was as follows: I created batches of eggs and measured their sizes. After the eggs hatched, I picked a subset of larvae from each egg clutch, and gave it to a foster female, that was of a standardised age and size. The female was later killed and used as a stimulus in order to observe the begging behaviour of the larvae. The broods were then monitored until they dispersed from the carcass to account for differences in their growth and survival based on their original egg size. Methodological limitations did not allow me to examine the relationship between egg size and behaviour at an individual level, as there is essentially no realistic method for marking individual larvae. Therefore I used brood means as measures for all traits examined. Details for the specific experimenta procedures at different stages of the experiment are described below. All beetles used in this experiment originally derived from wild caught beetles trapped in Craiglockhart hill in Edinburgh (UK), and in Warmond (Netherlands) (for details on husbandry and housing conditions, see Chapter 2).

3.2.1.1 *Egg clutch creation and egg measurements*

I aimed to attain as wide a range of egg sizes as possible within the scope of the natural size variation in the beetle population. To this end, I paired beetles of known size to other beetles of corresponding sizes in attempt to use the body size of the parents to produce eggs from both the small and large end of the distribution. Bigger mothers have been shown to lay larger eggs (Steiger, 2013), so therefore a relationship between parental size and egg size can be expected. Thus, prior to mating the beetles, I measured the length of the adult beetles' pronotum with a Mitutoyo absolute digimatic caliper (1-150 mm). The length of the pronotum is a good measure for the size of the beetle, as it does not change with time elapsed since it's last feeding, and is highly repeatable (Beeler et al., 1999). I divided the beetles into rough categories, classifying the beetles above the 75% quartile range as large (range: 4.54 - 4.87 mm), and below 25% quartile as small (range: 2.62 - 4.15 mm), leaving the rest of the beetles

classified as medium sized (range: 4.16-4.59 mm). I then selected pairs of nonsibling virgin male and female beetles within each size class to be mated together, with the expectation that the small pairs would produce eggs from the small end of the natural size variation, and the large pairs would produce large ones. A total of 203 pairings were conducted, out of which 48, 128, and 27 were in the small, medium and large categories, respectively. All matings were conducted in transparent containers (12 x 18 x 6 cm) filled with 1 cm of moist soil and a previously frozen mouse carcass to breed on (range 21- 26 g, supplied by Livefoods Direct Ltd, Sheffield, UK). The ages of the beetles were controlled for, and all beetles were mated 10-30 days after they eclosed as adults.

The pairs of beetles were given 60 hours to mate, prepare the mouse, and for the female to lay eggs in the soil. The parents were then removed from the egg boxes, and all medium sized females and their respective mouse carcasses were moved to empty containers filled with moist soil. All males, and the females of the small and large category were discarded. I only used the medium sized females as foster parents, to control for the confounding effects of female size on offspring growth, as female size has been shown to correlate with the amount of care given (Steiger, 2013). Males were discarded, as male care is highly variable, and has no detactable effects on the survival of the offspring produced (Eggert et al., 1998; Smiseth et al., 2005). The majority of the eggs laid in the soil of the mating boxes are situated at the bottom of the containers, and are visible through the transparent plastic (Figure 3.1). By counting the number of eggs visible at the bottom of the containers, I acquired an estimate for the total number of eggs laid, which correlates strongly with the actual clutch size (Monteith et al., 2012). After the eggs were counted, I scanned the egg boxes with a Canon CanoScan 9000F Mark II flatbed scanner, and the digital images (Figure 3.1) were then used to measure the sizes of the eggs.

I measured the eggs using Image J image processing program (Schneider et al., 2012). Only eggs that were laying flat against the surface of the box were measured, avoiding tilted eggs or those that were only partially visible in the soil, as such eggs would provide inaccurate measures. I measured the length and width of each egg, from the outermost tips of each specimen, using 300% magnification. I then calculated the volume V for each egg using the equation $V = (1/6)\Pi w^2 L$, where w is the width and L the length of the egg (Berrigan, 1991). A brood mean was then calculated from each egg measured in a brood. I

Figure 3.1: An example of a scan of an egg box, where the egg measurements were taken. Only eggs laying flat against the surface, and fully visible were measured.

checked the possibility of skewed distributions within broods of different clutch sizes or origins, by comparing brood means to brood medians. All brood means corresponded with the brood medians well, indicating that a brood mean is a reliable measure of average egg size within a brood across all broods measured.

3.2.1.2 *Behaviour measurements*

The egg boxes were checked for hatching six times each day. After hatching, I picked 10 larvae from each brood, weighed them with a digital scale (Ohaus Pioneer, with an accuracy of 0.1 mg) to attain their initial brood masses, and gave them to a foster female. Only females whose own eggs had started hatching were selected as fosters to avoid filial cannibalism (Müller and Eggert, 1990). The foster female was then allowed to raise the larvae undisturbed for 24 hours. I recorded larval begging 24 h (+/- 15 minutes) after the broods had been given to the females. This timing coincides with the peak in larval begging behaviour (Smiseth et al., 2003, Chapter 2). Thirty minutes before starting the observation, the female was removed in order to prepare it for it's use as a stimulus for recording larval begging. I killed the female by placing it in a -20°C freezer for 20 min, after which it was left to thaw for another 5 min before pinning the female onto a small plastic container (12 x 8 cm x 2 cm) lined with a moist paper towel. The same methodology has been used previously for recording larval begging in a variety of studies (see for example Leigh and

Smiseth, 2012; Smiseth et al., 2010, Chapter 2), and it is especially useful in this study, as it eliminates any differences in begging due to parental behaviour towards the larvae. The beetle was pinned in a position mimicking that of a parent regurgitating food. Once the beetle had been pinned, I removed the larvae from the mouse carcass, and placed them next to the pinned beetle. I waited 5 min before starting the observations to give the larvae time to settle. I recorded larval begging using instantaneous recording every 1 min during a 30 min observation period according to established protocols (Smiseth et al., 2010). The proportion of time spent begging by each larva in the brood, B, was calculated as $B = (b/l)/30$, where b is the total number of begging events during the 30 min observation period, and l is the mean number of larvae near the adult during each scan (i.e., within 0.8 cm diameter from the pin). Not all broods that were initially set up could be used to attain behaviour data, partially due to hatching failure, and time limitations imposed upon by the behaviour observations. The total sample size amounted to 97 broods, with the majority of the broods originating from the pairings between parents in the medium sized class (n = 21, 57, and 19, for the small, medium and large, respectively).

3.2.1.3 *Larval growth and mortality*

After the observations, the larvae were weighed, counted, and returned to the carcass to complete their development until dispersal. They received no parental care for the rest of their development, because the foster female that had been caring for them prior to the experiment had been killed. I did not substitute the foster female with another female beetle, as larvae of this species have been shown to beg more towards unfamiliar caring beetles (Chapter 2), and while the reason for it is still unclear, I wanted to avoid any potential confounding effects arising from it. By raising the larvae without the parent through the rest of their development, I was also able to measure their independent self-feeding ability in comparison to their begging intensity. Therefore, I was able to compare their survival and growth during a period of parental influence, and after it. I checked the boxes daily to record the timing of dispersal or death of all larvae in the brood. At dispersal I counted the number of larvae surviving, to account for mortality during the juvenile period, and weighed the broods once more.

3.2.2 *Statistical analyses*

All analyses were conducted with R version 3.1.2 (R Core Team, 2013). I used generalised linear mixed effects models (package lme4, Bates et al., 2014) for traits with gaussian error distribution (egg volume, clutch size, larvae size, egg development time, larvae development time), and generalised linear mixed effects models (package glmmADMB, Fournier et al., 2012; Skaug et al., 2014) for traits with negative binomial (larval mortality) or beta error distribution (larval begging). In all models, the size class of the biological parents of the eggs was used as a random variable. The specific structures for models for each trait analysed is described below. After the initial models were defined, non-significant ($P > 0.1$) terms were removed based on ANOVA's comparing the maximum likelihood estimates of the nested models in order to attain parsimonious models.

Larval behaviour

In analysing the proportion of time each larva within a brood spent begging, I assigned egg volume and the size of the larvae at observation time as fixed factors. I also added the size of the foster female as a covariate, as even though the size was controlled for experimentally, it may still affect the amount of begging within the size range of the medium sized females. I also added the two-way interaction between foster female size and larval size at the time of the observation (24 hours) as a covariate, as the bigger mothers may provide more care, and thus enhance the growth of their offspring (Steiger, 2013).

Egg traits

For analyses on egg traits (egg volume, clutch size, and egg development time), I set female pronotum length and male pronotum length, and the two-way interaction between the two as fixed factors. To then assess whether egg volume affected either clutch size or development time, I added volume, and the two-way interactions between volume and parental sizes (female size: volume, male size: volume) as covariates in these models. Similarly, clutch size and its corresponding interactions were added to the models for egg volume and development time. As within brood variation in egg size might be different for broods of different mean egg volume, i.e. larger eggs might be produced at a cost to the size of other eggs in the broods, or smaller eggs may come from broods with more homogenous size distribution, I also added within brood

standard error of egg volume, and two-way interactions between this variation measure and parental sizes into the models for egg volume and development time. The measure of variation was not added to the analysis on clutch size, as the number of eggs measured was used to calculate standard error, and as it correlates strongly with the total number of eggs in a brood, the two variables would not be independent.

Larval size and mortality

The size of the larvae was analysed at three different timepoints. Firstly, in the models for larval size at hatching, I added egg volume and egg development time and the two-way interaction between the two as fixed factors. Secondly, in analysing larval size at the time of the observations (24 hours after hatching), I also added larval size at hatching, as well as it's interaction with egg volume, into the model. Thirdly, in analyses on larval size at dispersal, I further added larval size at the time of the observation and it's interaction with egg volume into the model. I also added the proportion of time spent begging, number of larvae at dispersal, and larval development time into the model. I also analysed larval development time from newly hatched larva to dispersal. In this model, I assigned egg volume, egg development time, the three measures of larval size, proportion of time spent begging, number of larvae at dispersal and foster female size as fixed factors. For analysis on larval mortality, I used the count of larval deaths from the start of the experiment, when a brood of 10 newly hatched larvae was created, to dispersal as a response variable, and assigned egg volume and the proportion of time spent begging by a larva in a brood along with a two-way interaction between the two as fixed factors. I assigned the size of the foster female, and larvae size at dispersal and the interaction between the two as covariates, to account for differences in care based on foster female's size (Steiger, 2013), and the size of the offspring themselves.

Table 3.1: The association between egg volume and begging behaviour in *Nicrophorus vespilloides*. For each factor, I present parameter estimates (Par), standard errors (SE), test statistics (z-value), and *P*-values. All estimates are derived from a generalised linear mixed effects model (glmmadmb), where the error structure was modelled with a beta distribution.

Factor	Par	SE	z-value	*P*-value	
Egg volume	-23.13	12.25	-1.89	0.059	.
Foster female size	-10.25	6.03	-1.70	0.089	.
Egg volume: Foster female size	5.13	2.78	1.85	0.065	.

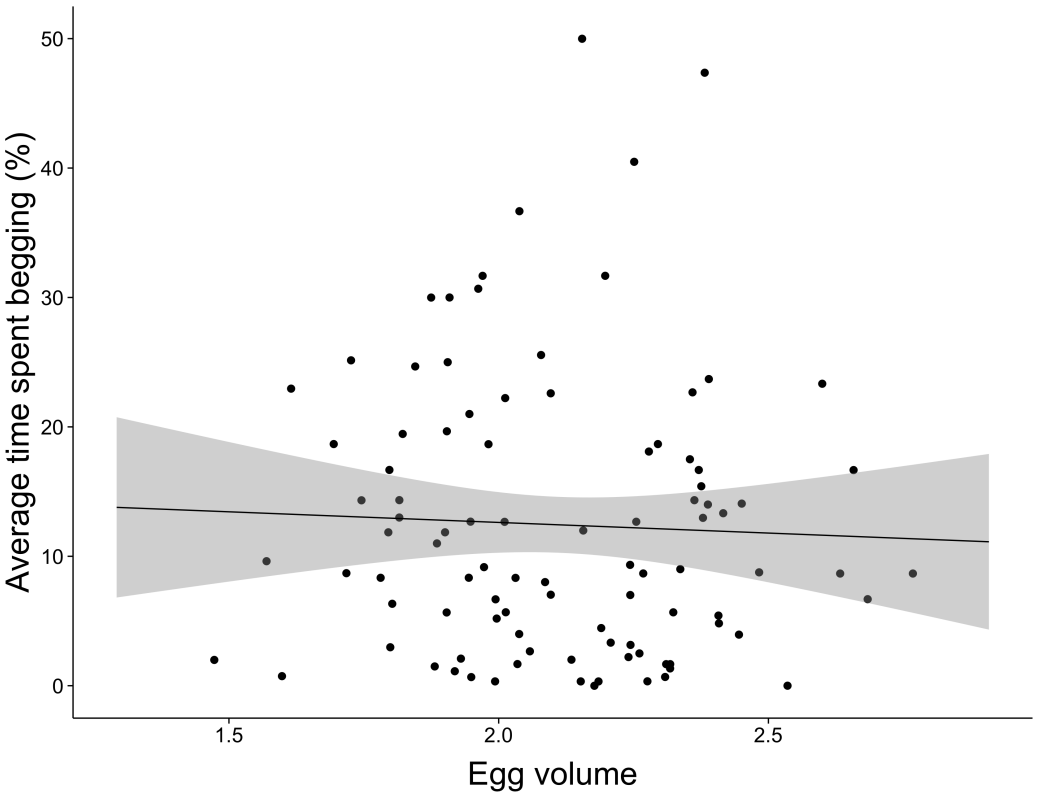


Figure 3.2: The association between egg volume and begging behaviour in *Nicrophorus vespilloides*. Begging is presented as percentage of time an average larva spent begging in a brood during the 30 minute instantanious scan sampling period, conducted as the larvae were 24 hours old.

3.3 Results

3.3.1 *Relationship between egg volume and begging*

There was a lot of variation in both egg volume and the average time spent begging by a larva in a brood (Figure 3.2). Begging mostly occured at relatively low levels (mean +/- standard error; 12.4 +/- 0.8 % of the time measured), comparable to other studies with a similar setting for measuring begging behaviour (Leigh and Smiseth, 2012; Smiseth et al., 2010, Chapter 2). However, there was no significant relationship between egg size and begging behaviour (Table 3.1).

Table 3.2: The association between egg traits (volume, number (i.e. clutch size), and development time) and parental sizes in *Nicrophorus vespilloides*. For each trait I present parameter estimates (Par), standard error (SE), degrees of freedom (df), test statistics (t-value), and *P*-values. All estimates are derived from a linear mixed effects model (lmer), with degrees of freedom and *P*-values attained through Satterwaithe approximation.

Response	Factor	Par	SE	df	t-value	*P*-value	
Egg volume	Female size	3.94	1.18	120	3.34	0.001	**
	Male size	3.90	1.22	120	3.21	0.002	**
	Clutch size	0.01	2.13 x 10^{-3}	120	3.46	<0.001	***
	Female size: Male size	-0.90	0.28	120	-3.17	0.002	**
Clutch size	Egg volume	13.21	3.27	21	4.05	<0.001	***
Egg development time	Egg volume	-0.80	0.14	116	-5.92	<0.001	***
	Egg size variation	-2.27	1.28	116	-1.78	0.078	.

3.3.2 *Effects of parental size on egg traits*

Both the size of the female and the size of the male affected egg size, and the interaction between the sizes of the parents was significant (Table 3.2). Variation in size traits for both parents was large (Figure 3.3). The relationship between clutch size and egg volume indicated a positive correlation between the two traits, as clutch size was positively associated with egg volume, and similarly egg volume was positively associated with clutch size (Table 3.2, Figure 3.3c). Egg development time was affected negatively by egg volume (Table 3.2), indicating that larger eggs developed faster than the smaller ones. Egg development time was not affected by the size of either of the parents.

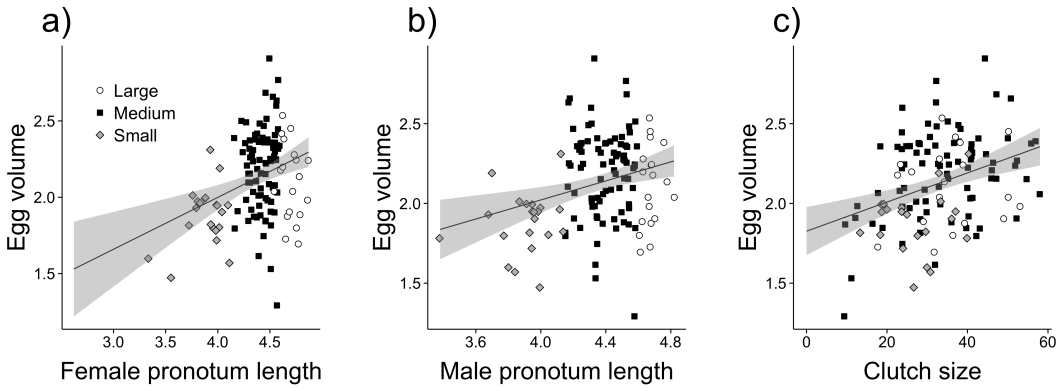


Figure 3.3: Factors affecting egg volume in *Nicrophorus vespilloides*. Comparison of egg volume and (a) size of the female parent, (b) size of the male parent, and (c) clutch size. The shaded area indicates the standard error confidence band for the slope. Parental size class is indicated by different symbols representing the large beetles (4.54 - 4.87 mm), medium sized beetles (4.16-4.59 mm), and small beetles (2.62 - 4.15 mm).

Table 3.3: The effects of egg volume on larval size, development time, and survival in *Nicrophorus vespilloides*. For each trait I present parameter estimates (Par), standard error (SE), degrees of freedom (df, not applicable for analysis on larval survival), test statistics (t-value for larval size traits and development time, z-value for the number of deaths), and *P*-values. Estimates are derived from linear mixed effects model (lmer), with degrees of freedom and *P*-values attained through Satterwaithe approximation (larval size measures and development time), or from a generalised linear mixed effects model (glmmadmb; number of deaths).

Response	Factor	Par	SE	df	t/z-value	*P*-value	
Larval size at hatching	Egg volume	6.00 x10^{-4}	1.34 x10^{-4}	135	5.48	<0.001	***
	Egg development time	1.83 x10^{-4}	6.20 x10^{-5}	136	2.96	0.004	**
Larval size at 24 hours	Egg volume	-3.45 x10^{-3}	1.65 x 10^{-3}	94	-2.09	0.0389	*
	Larval size (hatching)	7.48	1.61	94	4.65	<0.001	***
Larval size at dispersal	Larval size (hatching)	18.81	7.27	86	2.59	0.011	*
	Larval size (24 hours)	0.91	0.47	85	1.96	0.054	.
	Brood size (dispersal)	3.21 x10^{-3}	8.33 x 10^{-3}	85	3.85	<0.001	***
	Larval development time	-6.87 x10^{-3}	1.87 x 10^{-2}	85	-3.67	<0.001	***
Larval development time	Larval size (dispersal)	-20.03	4.77	81	-4.20	<0.001	***
	Brood size (dispersal)	0.13	0.04	81	2.95	0.004	**
	Egg development time	0.57	0.20	82	2.87	0.005	**
Number of deaths	Begging	65.95	39.26	-	1.68	0.093	.
	Egg volume	0.04	0.78	-	0.05	0.958	
	Foster female size	5.74	1.55	-	3.71	<0.001	***
	Larval size (dispersal)	-17.85	5.58	-	-3.20	0.001	**
	Begging: Egg volume	8.52	4.60	-	1.85	0.064	.
	Begging: Foster female size	-18.92	8.96	-	-2.11	0.035	*

3.3.3 *Effects of egg volume on larval growth and mortality*

Egg volume determined larval size at hatching, but its direct effect disappeared towards the end of larval development (Table 3.3). Egg volume still had a statistically significant impact, albeit a negative one, on larval size at 24 hours after hatching (Table 3.3). To assess whether this effect was only due to its influence on larval size at hatching, or whether egg volume had an independent effect on larval size at 24 hours after hatching, I conducted a post-hoc analysis comparing the model shown in Table 3.3, and a similar model without larval size at hatching as a factor. The post-hoc test shows that the effect of egg volume lost its statistical significance after the removal of larval size at hatching (lmer: t_{95}=0.02, P=0.983), therefore indicating that this effect was mediated through larval size at hatching. A negative effect could potentially indicate slight differences in the growth of the larvae originating from different-sized eggs, where larvae from smaller eggs grew faster, thus evening out the differences in size to a small degree. Larval size at hatching also affected size at dispersal, with which larval size at 24 hours also had a marginally non-significant positive association, but egg volume had no effect (Table 3.3).

To assess whether the effects of larval sizes at different time points had an independent effect on size at dispersal, I conducted post-hoc tests of removing either of the size variables from the models to see if they caused changes in the other. When larval size at 24 hours was removed, the effect of larval size at hatching remained the same (lmer: t_{87}=3.82, P <0.001). However, when larval size at hatching was removed, larval size at 24 hours had a statistically significant effect on larval size at dispersal (lmer: t_{85}=3.35, P=0.001), indicating that the effect of larval size at 24 hours was originally masked by the stronger effect of larval size at hatching in the previous model. Brood size had an effect on the size of the larvae at dispersal, with the larvae being slightly larger in broods with more surviving larvae (Table 3.3).

Larval development time (i.e. time to dispersal) was associated with larval size at dispersal, with the bigger larvae developing faster than smaller ones (Table 3.3). Brood size at dispersal also had a positive effect on development time (Table 3.3), indicating that the more larvae survived to dispersal, the faster they developed. Both of these effects potentially indicate that there are quality differences correlated with size differences in the larvae. Larval development time was also positively affected by egg development time, as the faster the eggs developed, the faster the larvae reached dispersal (Table 3.3), indicating that large individuals are fast in their development both in egg phase and in the later juvenile phases. Larval mortality was affected by larval size at dispersal, with larger larvae having lower mortality during larval development (Table 3.3). Time spent begging, and size of the foster mother also affected larval mortality, though their effects were dependent one another, ultimately improving larval survival, as apparent in the significant interaction term (Table 3.3). An interaction term between begging and egg volume that was marginally non-significant was also retained in the model, so therefore the effects of other variables were corrected by the interaction between the two traits (Table 3.3).

3.4 Discussion

The main aim of this study was to investigate the association between egg volume and offspring begging behaviour in the burying beetle, *Nicrophorus vespilloides*. I found no evidence for a relationship between these two traits. I

did, however, find evidence for a positive association between egg size and offspring growth and development: bigger eggs were found to develop faster, larger eggs became larger larvae at hatching, which in turn affected size at dispersal. Burying beetle larvae do not feed after they disperse from the carcass, and therefore larval size at dispersal is a good predictor of larval size as an adult (Bartlett and Ashworth, 1988; Lock et al., 2004). Adult body mass in turn is a good predictor of reproductive success, as it influences success in competition for carcasses (Otronen, 1988). I found that larval size at dispersal was also negatively associated with larval mortality, indicating that large larvae had higher survival. My results suggest that, while egg size has no impact on begging behaviour, and thereby is unlikely to affect the amount of post-hatching care the offspring receive, egg volume improves offspring fitness in other ways. Correlations between egg size and traits affecting offspring fitness have been found in numerous other studies, as reported in a meta-analysis on avian literature (Krist, 2011). My results provide an important addition to the current knowledge regarding the association between egg size and fitness traits, by excluding offspring begging behaviour from the pool of potential mechanisms giving rise to these correlations.

Finding no relationship between egg size and begging contradicts my initial prediction of parental care masking the initial effects of egg size due to differential begging exhibited by larvae originating from different sized eggs (Monteith et al., 2012). Therefore it seems plausible that the masking effect of parental care does not require for the parent to adjust the amount of post-hatching care they provide to compensate for the potential effects of laying small eggs, and are consistent with the suggestion that the strong effects of post-hatching care mask the much smaller effects of egg size (Monteith et al., 2012). Post-hatching parental has evolved to neutralise a variety of environmental stressors, such as predation, paratisism, competition and starvation (Royle et al., 2012), and it can also buffer against inbreeding depression in the burying beetles (Pilakouta et al., 2015). Post-hatching care therefore provides a powerful means for improving offspring fitness by evening out any adverse effects derived from their environment or internal state, and has much stronger effects on larval growth than egg size (Ricklefs, 1984). Begging could also not expain the higher levels of post-hatching care provided by bigger mothers (Steiger, 2013). Therefore it can be presumed that the difference in the parental care provided by large and small females is due to variation among

parents (Steiger, 2013), and not merely a result of parents responding to offspring signals of need. I cannot, however, rule out the possible effects of egg volume on begging during the first few hours after hatching. In birds, egg components have been shown to affect begging only early in the development (Schwabl, 1996). However, I conducted the observation at 24 hours after hatching, because this corresponds to the peak in larval begging, when larval need is at its highest (Smiseth et al., 2003, Chapter 4). Should larvae originating from different sized eggs beg differently, it would be expected for these effects to be apparent at the peak time of need in the period of larval dependency, especially as in my data egg size still has an effect on the size of offspring at the time. Therefore despite the potential effects of egg size on begging during the first few hours after hatching, I argue that my results suggest that the benefits from post-hatching care do mask the effects of egg volume (Monteith et al., 2012), and bigger females do provide higher levels of care (Steiger, 2013), and neither phenomena are driven by offspring begging behaviour.

Another implication of our result regarding the lack of a relationship between egg volume and offspring begging, is that it adds to our understanding of the nature of begging behaviour as a honest signal of need (Kilner and Johnstone, 1997). In the burying beetle, begging reflects larval hunger state, as starved larvae beg at higher levels than those that have been provided with ample amounts of food (Andrews and Smiseth, 2013; Rauter and Moore, 1999; Smiseth and Moore, 2004a). Offspring needs can, however, be divided in two categories: the short-term needs, or the long-term needs (Price et al., 1996). Hunger represents offspring's short-term needs, while egg size would contribute towards the offspring's long term needs, which represents the amount of resources necessary for completing offspring development up to independence from the parents (Price et al., 1996). In yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), when short-term needs were controlled for, chicks begged at different levels based on their condition, indicating that begging reflects the offspring's long term needs. However, it is currently unclear whether egg size influences the offspring's long term needs: egg size may be linked with fitness traits relating to offspring quality, as suggested both by literature (Krist, 2011) and my data. However, in a species where the offspring can self-feed, it is possible that the reward from begging based on the long term needs do not necessarily result in a benefit large enough to merit engaging in a costly behaviour, such as begging, given that it increases the risk of becoming a target

of filial cannibalism (Andrews and Smiseth, 2013). Contrary to my results on egg size, there is evidence that egg composition, laying order as well as the hormone levels associated with it, can affect offspring behaviour or performance in some species of birds and fish (Fuiman and Ojanguren, 2011; Gilby et al., 2012; Rice et al., 2013). It is possible that these traits are more connected to the physiological processes of offspring development, which in turn bears severe implications to the later life of the offspring (i.e. Bateson et al., 2004; Kilner et al., 2015; Nussey et al., 2007), thus potentially influencing their decisions to beg more severely.

My results provide evidence for a positive association between egg size and offspring quality due to the finding that larger eggs did not only produce larger offspring, but that these eggs also developed faster. Eggs that developed faster in turn created larvae that reached dispersal faster. Ultimately, there was also a positive association between size at dispersal and survival. Intrisic properties of eggs have been found to have a positive effect on the development time of embryos also in zebra finches *Taeniopygia gluttata* (Griffith and Gilby, 2013). However, a study on a congeneric species, *Nicrophorus quadripunctatus* found no association between egg volume and embryonic development time (Takata et al., 2015). It is possible that the difference between the two studies are due to differences between the two species, as *N. vespilloides* larvae survive without parental care provided they have a food source, whereas *N. quadripunctatus* larvae are dependent on parental care for the first day after hatching, making parental care obligatory for the survival of the larvae in the latter (Satou et al., 2001). Therefore *N. vespilloides* may receive more benefits from producing large eggs of a higher quality, potentially due to variation in the amount of care provided, or the quality of the food source: If both parents would happen to abandon the nest, the high quality offspring might still survive given that they would find the carcass to be of a suitable quality in *N. vespilloides*, but would be most likely to die in *N. quadripunctus*. A more likely explanation, however, is that egg size, and therefore larval size at hatching, may also be linked to larval self-feeding ability, which improves over their juvenile phase (Smiseth et al., 2003). If large larvae are better at self-feeding, their faster development to dispersal is likely to be explained by it, as in my study, the parent was absent after 24 hours after hatching. As large size has been shown to have positive effects in multiple traits of the adult beetles (Otronen, 1988; Steiger, 2013), a positive association between multiple offspring fitness traits and egg volume

provides further evidence for the much researched topic of positive association between fitness and body size (reviewed in, for example, Kingsolver and Huey, 2008).

The link between egg size and offspring fitness traits bears implications for our understanding of the maintenance of variation in egg size even in species with elaborate post-hatching care. Even when the effects of egg size can be masked by the much stronger effects of post-hatching parental care (Monteith et al., 2012), there are still benefits for producing large eggs. In *N. vespilloides*, offspring quality declines with the timing of the mother's first reproduction, which in turn is largely determined by chance due to the scarcity of resources (Lock et al., 2007). A plastic response in the amount of care provided can thus ameliorate the adverse effects of first reproduction that happens past the optimal age (Lock et al., 2007). Competition for carcassess is also harsh (Otronen, 1988), and brood paratisism is relatively common (Müller et al., 1990), making the environment in which the beetle larvae are raised harsh. The relative advantages of producing larger eggs have been shown to be greater in harsher environments (Fox and Czesak, 2000), thus making procuding large eggs a viable strategy for *N. vespilloides*. Fast developing eggs are potentially good at competing against brood parasites, as well as at being parasites themselves, suggesting a benefit from producing large, good quality eggs. Therefore, fast growth associated with large eggs has implications to the fitness of the offspring even in a species which provides mostly post-hatching care. Because *N. vespilloides* is a species with partial begging, these benefits could prove important links in understanding the coevolution of egg size and parental care.

The finding that larger eggs developed faster both as eggs and as larvae also has implications to the evolutionary models regarding the coevolution of egg size and parental care (Gilbert and Manica, 2010; Nussbaum, 1985, 1987; Nussbaum and Schultz, 1989; Sargent et al., 1987; Shine, 1978, 1989). Firstly, this fast development throughout different phases violates the assumptions of the safe harbour hypothesis, which suggests that the larger eggs spend more time in the egg phase in order to minimise the time spent at the juvenile phase (Shine, 1978, 1989). Secondly, it is possible that the faster development may be an adaptive response to higher egg mortality of larger eggs in this species, as predicted by the alternative theoretical model for the coevolution of egg size and parental care (Nussbaum, 1985; Nussbaum and Schultz, 1989). Eggs of the

burying beetle are laid in the soil, and therefore larger eggs may potentially be more vulnerable to predation by other insects or disease caused by fungi due to their increased visibility and larger surface area. Thirdly, the relationship between egg size and offspring quality, combined with prior evidence showing that maternal size affects the amount of care given (Steiger, 2013) could potentially suggest that the coevolution of egg size and parental care is an adaptive life history strategy for coping with harsh environments driven by resource availability (Itô and Iwasa, 1981). The coadaptation between the two traits could potentially explain our results regarding the interaction between begging behaviour and foster female size having a positive effect on larval survival. It is possible that this effect arises due to a mismatch between the parents and the offspring, as both the amount of parental care and offspring begging are dependent on maternal state. Thus, when maternal state and the state of the mother of the foster brood do not match, larval survival is affected (Lock et al., 2007). Therefore the interaction between foster female size and begging behaviour could potentially indicate co-adaptation between the two traits. However, further studies are needed to adress the potentially adaptive nature of the relationship between egg size and egg fitness traits in relation to parental behaviour, to fully understand it's implications to the coevolution of parental care and egg size.

Chapter 4

Control over parental care is a compromise between the parents and the offspring

Abstract

Parents and offspring have different optima for the level of parental resource allocation and the timing of nutritional independence. Previous work has focused mostly on either parental or offspring traits, but work combining both traits is still lacking. Burying beetle *Nicrophorus vespilloides* provides elaborate parental care, and the larvae solicit food from their parents with conspicuous begging displays. Larval begging peaks 24 hours after hatching, after which they become more proficient at feeding themselves and spend less time begging. Begging ceases altogether 72 hours after hatching, which marks the transition to nutritional independence. Here I present data from an experiment investigating the expression of both offspring and parental traits from hatching to transition to independence. By manipulating the age of the brood the female was caring for, I created mismatch in the perceived optimal provisioning rates between the perspectives of the parent and the larvae. I find that the females adjusted the total amount of provisioning based on the needs of the larvae, but both the parents and the offspring influenced the realised levels of provisioning, which fully followed neither parental preferences nor offspring need. My results suggest that the resolution of the power struggle is a compromise.

4.1 Introduction

Parents and offspring have different optima for the level of parental resource allocation and the timing of nutritional independence, giving rise to a conflict between parents and offspring (Godfray, 1995a; Parker and Macnair, 1979; Trivers, 1974). In many taxa, including mammals, birds, amphibia and insects, the resolution of this conflict is mediated by elaborate offspring begging displays that offspring use to elicit food from their parents (Kilner and Johnstone, 1997). Both the parents and the offspring have the potential to bias the level of resource allocation towards their own optima thus providing them with some

control over the amount of care given (reviewed in Kilner and Johnstone, 1997. Empirical studies, conducted mainly on altricial birds, provide support for either parental (e.g.Kilner and Johnstone, 1997; Kölliker et al., 1998; Rauter and Moore, 1999 or offspring control (eg. Parker et al., 2002b; Rodríguez-Gironés et al., 2001; Smiseth et al., 2003 over the level of parental resource allocation. However, this simplistic dichotomy of either parental or offspring control may not accurately reflect the complexities underlying these interactions, which are through to involve a potential power continuum with the control shifting from offspring to parents during offspring development (Royle et al., 2002). Less is known about control over the duration of care, though evidence suggests that both parents and offspring have the ability to influence the timing of transition to nutritional independence: Offspring can extend the duration of care by begging more intensively over a longer period of time (Kilner and Drummond, 2007), and parents can reduce the length of the juvenile period by reducing their responsiveness to begging (Davies, 1976; Thorogood et al., 2011), showing aggression towards the offspring (Leonard et al., 1991), or by abandoning the brood (Reiter et al., 1978). Studies investigating control over parental care by combining information on both the level of care and its duration are still lacking.

For both parents and offspring, the optimal level and duration of care reflect changes in the benefits and costs of providing and receiving care throughout offspring development (Hinde et al., 2010; Royle et al., 2002). These changes lead to age-dependent coadaptation in parental supply and offspring demand, which are expressed as matches between the behaviour of parents and offspring at a given offspring age and parental reproductive stage (Gómez and Kölliker, 2013). Little empirical work has been done to study the effects of disrupting such age-dependent coadaptation between parents and offspring. Riou et al. (2012) studied the transition to nutritional independence in Manx shearwaters using a cross-fostering design where younger and older chicks were swapped between nests to manipulate the age of the chicks that the parents were caring for. They found that the frequency of parental food deliveries and parental responsiveness to begging dropped at the later stages of development regardless of the level of begging exhibited by the chicks (Riou et al., 2012). The chicks exaggerated their signals of need, and their fledging was not affected by parental behaviours, indicating that the parents were in control of the level of food allocation but not the timing of nutritional independence (Riou et al., 2012). However, the study relied on a single manipulation during the caring period.

Repeating the age-manipulations throughout the caring period would provide more conclusive insights into the potential changes in the pattern of parental or offspring control over the amount and duration of parental care.

The burying beetle, *Nicrophorus vespilloides*, is an excellent study system for investigating the resolution of parent-offspring conflict, as the adult beetles exhibit elaborate parental care for their larvae, which they raise on carcasses of small vertebrates (Eggert and Müller, 1997; Scott, 1998). Although the larvae are capable of feeding from the carcass prepared by the parents on their own (Eggert et al., 1998), they also beg for food from their parents by touching the adult with their feet, after which the parents regurgitate pre-digested carrion for them to feed on (Smiseth and Moore, 2002; Smiseth et al., 2003). Both parents are involved in parental care, although usually only the females stay with the brood until the larvae disperse from the carcass into the soil to pupate (Scott, 1998). Begging in the burying beetles reflects the hunger levels of the larvae, and is as such considered to be an honest signal of need (Smiseth and Moore, 2004a, 2007; Smiseth and Parker, 2008). Larval begging behaviour changes over time as the larvae become more proficient in self-feeding (Smiseth et al., 2003). The behaviour peaks at 24 hours after hatching, and starts declining after that, until approximately 72 hours after hatching, which marks the point of transitioning to nutritional independence (Smiseth et al., 2003). Larvae also beg differently towards different classes of adult beetles, exhibiting discrimination against non-breeding individuals (Chapter 2), suggesting that they have the ability to adjust their behaviour based on cues they receive from their parents.

In this experiment, I investigated whether the parents or the larvae were in control of parental care in terms of potential food allocation and the transitioning to nutritional independence in *N. vespilloides*. I created mismatch between the expectations of the parent and actual age of the offspring through repeated manipulations of the age of the brood that a female was caring for, thus creating treatments where the broods remained at the early, mid, or late stage of juvenile development rather than aging naturally. My aim was to explore the patterns of potential food provisioning and begging in these mismatched treatments in comparison to a matched one, where the larvae aged according to parental expectations. The larvae of different ages beg at different intensities in a natural setting (Smiseth et al., 2003), and as such the response of the parent in the mismatched treatments reflects whether they base their

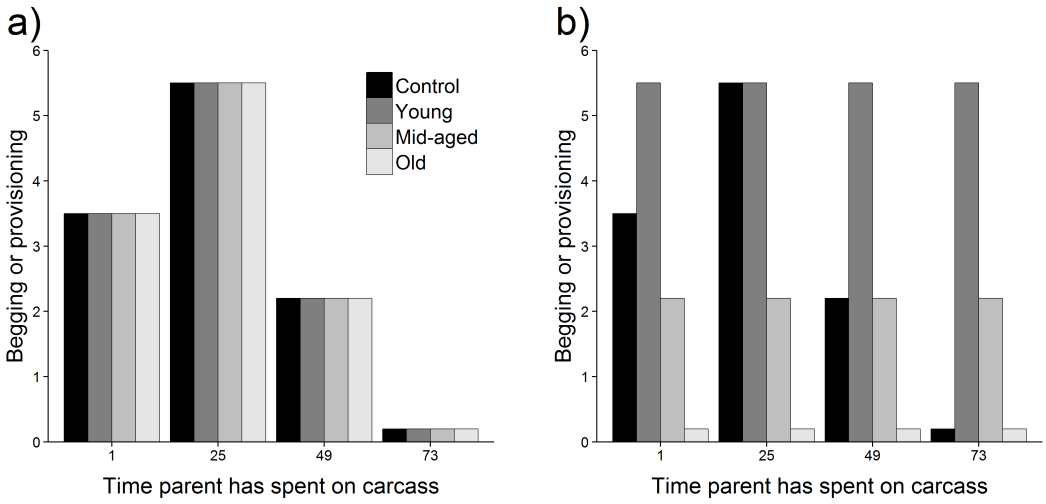


Figure 4.1: Expectations for the pattern of begging and provisioning based on assumptions of a) full parental control, and b) full larval control. Pattern drawn after Smiseth, Darwell & Moore 2003.

behaviour on cues they receive from the larvae or cues arising from their own perception of the age of the brood. If the parent is in control, it should provision food based on their own expectations regardless of the changes in larval age, and thus all treatments would follow a pattern that corresponds to the pattern of the matched (i.e. control) treatment (Figure 4.1a). If the larvae are in control, the parent should adjust its behaviour based on cues of larval need, and the levels of food provisioning would remain at the level determined by larval age (Figure 4.1b). If neither the parent nor the offspring are in full control, the pattern of food provisioning would reflect a compromise, where the realised levels of provisioning would lie between the two extremes. To explore mechanisms through which either the larvae or the parents can take control over parental care, I also explored patterns of other behaviours likely to be affected by parent-offspring conflict. Thus, in my investigation I examined i) total food provisioning provided by the parent, ii) larval begging and parental responsiveness to it, iii) the total amount of care provided by the parent, and iv) the size and age of the larvae at dispersal.

4.2 Materials and methods

4.2.1 *Experimental design*

The beetles in the experiment were a part of an outbred laboratory population originating from wild caught beetles trapped in Corstophine Hill and Craiglockhart Hill (Edinburgh, UK), Kennall Vale, (Cornwall, UK) and Madingley Woods (Cambridge, UK) (for details on the housing conditions of the beetles, see Chapter 2).

I randomly selected pairs of nonsibling virgin male and female beetles to be mated. The pairs were moved to a transparent container (12 x 18 x 6 cm) filled with 2 cm of moist soil and a previously frozen mouse carcass (range 20-25 g, supplied by Livefoods Direct Ltd, Sheffield, UK). Male care is highly variable and has no detectable effects on larval growth or survival under laboratory conditions (Eggert et al., 1998; Smiseth et al., 2005), and therefore I only used female parents in this experiment. I removed the male 60 hours after pairing before the larvae started hatching. Concurrently, I moved the female and the carcass into a new container filled with soil in order to separate the eggs from the breeding female. The egg boxes were checked 5 times a day for hatching.

I created mismatch between the actual age of the brood and the age the parent expected it to be by repeated cross-fostering throughout larval development. Age manipulations were conducted by swapping the brood that an experimental female was caring for, with another experimental brood of a known age every 24 hours for the approximate duration of larval dependency (i.e. first 72 hours after hatching). In order to achieve this, I needed a supply of larvae of known ages throughout the experiment. To this end, I created donor broods consisting of larvae of an appropriate age, and cared for by a non-experimental female foster parent. For each experimental female, I created 2-3 donor broods to ensure access to an excess number of larvae. The donor broods for a given experimental female were created every 24 (+/- 15 min) hours over 4 consecutive days. I picked 15-25 newly hatched mixed maternity larvae for each donor brood, and moved these broods into a container with a female parent and a mouse carcass. I only used females whose own eggs had started hatching to avoid filial cannibalism (Müller and Eggert, 1990). The donor broods were used to create

experimental broods of 10 larvae at subsequent stages of the experiment.

I had four experimental treatments in this experiment, all of which followed the same general procedures: At the beginning of the experiment, an experimental female was given a brood of ten larvae of a known age. An hour later, I conducted a behaviour observation on the first brood, after which the brood was removed and replaced by another brood created from the appropriate batch of donor broods. All larvae taken away after the observations were returned into the pool of donors to be used to generate experimental broods later in the experiment for experimental females in other treatments. The observations were then repeated three times at 24-h intervals, with the larvae being swapped after each observation, aside from the last, after which the female was allowed to raise the larvae until they dispersed from the carcass. When the brood had dispersed, i.e. when all larvae had moved from the carcass to the soil around it, I removed the female and ended the experiment. I measured the length of the female's pronotum after her death with a Mitutoyo absolute digimatic caliper (1-150 mm).

My experimental treatments differed in the age of the brood that was used to replace the previous brood. (1) In the control treatment, the initial broods were set up using newly hatched larvae, observed as they were 1-hour old, and later the broods were always replaced by broods consisting of larvae of the same age as the ones taken away. This was done in order to control for the effects of swapping the broods. The broods were therefore observed as they were at the age of 1, 25, 49, and 73 hours, and the actual age of the brood always matched the parental expectations of larval age. In the rest of the treatments, the expectations of the parent and the actual age of the brood were mismatched by manipulating the ages of the broods as follows: (2) In the young treatment, the initial broods were set up with 24-hours-old larvae, the larvae were observed as they were 25-hours-old. After the observation, the brood was always replaced by a brood of 1-hour-old larvae, which were then observed 24 hours later as they were 25-hours-old. Thus the larvae remained at the early stage of the juvenile development for longer from the parent's perspective. (3) In the mid-aged treatment, the initial broods were created using 48-hours-old larvae, observed at the age of 49 hours, and afterwards always replaced by 25-hours-old larvae, which again were observed at the age of 49 hours. In this case, the larvae remained at the mid-stage of the juvenile period throughout the experiment. (4)

In the old treatment, the initial broods were created with 72 hour old larvae, observed as they were 73-hours-old, and after that always replaced with 49-hour-old larvae, which were observed 24 hours later at the age of 73 hours. Therefore the larvae were close to nutritional independence from the beginning to the end.

The behaviour observations consisted of 30 min of instantaneous scan sampling every 1 min (for details of the protocol, see Smiseth and Moore, 2002). At each scan, we counted the number of larvae begging and the number of larvae in mouth-to-mouth contact with the female (i.e. being provisioned for). I also noted whether the female was within a pronotum length's distance from the larvae, as larval begging is triggered only at close proximity (Rauter and Moore, 1999; Smiseth and Moore, 2002). I categorised the behaviour of the female at each scan into seven distinct categories: feeding the larvae, interacting with the larvae, guarding the larvae, maintaining the carcass, consuming the carcass, nonparental behaviours, and being away from the carcass altogether (see Smiseth and Moore, 2002 for definitions). The first four behaviours can be considered as forms of parental care, and as such I counted a measure of total care provided by the female by summing up the amount of time spent on these traits. Due to mortality in the donor broods, experimental females were occasionally discarded in the middle of the experiment, as there were no larvae to provide for them. The behaviour data from before the discarding was still used, leading me to have different sample sizes across all time points in the different treatments. My final sample sizes for each observations conducted at 1 h, 25 h, 49 h, and 73 h after the larvae were given to the female, were n= 21, 21, 21 and 20 for the control treatment; n = 39, 36, 35, and 25 for the young treatment; n = 26, 25, 20, and 19 for the mid-aged treatment; and finally n= 20, 19, 18, and 18 for the old treatment .

Based on the behaviour data, I measured total food provisioning provided by the parent, larval begging, parental food provisioning as a response to begging, and the total amount of care provided by the parent, details for which are discussed below. The full count of provisioning events within a behaviour observation (hereafter referred to as total provisioning) was used to explore the patterns of resource allocation in the matched and mismatched broods. This parental trait includes a number of observations where the female was not provisioning, both from when the parent was close to the larvae, and when it was away from them.

For investigations of larval begging, however, I excluded the data from when the parent was not close enough to the larvae to trigger begging. For this larval trait, I counted the total number of begging events within each observation, and it was used to explore whether larval behaviour was based on cues from the parents or their own state. I also counted another measure of provisioning, which was conditional to the parent being close enough to the larvae to experience begging (hereafter referred to as conditional provisioning), for which I thus only used the data when the parents were within a pronotum length's distance of the larvae. This parental trait only included observations where the female did not provision even though she was close enough to the larvae to beg from her, thus allowing me to explore parental responsiveness to begging. To account for changes in other types of care that the parent can provide, I calculated a sum of all caring behaviours within each behaviour observation (hereafter referred to as total care). The total sample sizes for the subset of the data that was used for begging and conditional provisioning in the different treatments were n = 14, 17, 15, 8 for the control treatment; n = 23, 23, 21, 7 for the young treatment; n = 21, 8, 14, 11 for the mid-aged treatment; and n = 17, 12, 17, 15. for the old treatment, for the observations at at 1 h, 25 h, 49 h, and 73 h after the larvae were given to the females, respectively.

4.2.2 *Statistical analyses*

All analyses were conducted with R version 3.1.1 (R Core Team, 2013). As the behaviour traits had zero-inflated negative binomial error structures, they were analysed using generalized linear mixed-effects models (R package glmmADMB, Fournier et al., 2012; Skaug et al., 2014. In all models, I assigned experimental treatment (control, young, mid-aged or old), time of observation (1 hour, 25 hours, 49 hours or 73 hours after the start of the experiment), and the interaction between the two, as fixed effects, and block and the identity of the female (to control for pseudoreplication) as random factors. I included the mass of the mouse as a covariate in the models, because the amount of resources available affects the parental decisions to care for the offspring (Smiseth and Moore, 2002). I also included brood size in the models, as it is known to influence begging behaviour of the larvae, and thereby it may also influence the parental traits (Smiseth and Moore, 2002; Smiseth et al., 2007b). Because begging reportedly increases with increasing brood size in a non-linear fashion

(Smiseth and Moore, 2002), brood size was log-transformed (to deal with non-linearity) in the model with begging as a response variable, and set as an offset variable to weigh the counts of begging against the number of larvae within the brood (Zuur et al., 2009). For all other response variables, brood size was simply included as a covariate in the models, as its influence over the other traits was not expected to be as direct. After the full models were fitted, the non-significant covariates were dropped in a step-wise simplification based on the maximum likelihood estimates obtained from ANOVAs between nested models, and only terms significant in P <0.05 level were retained in the models. Both the mass of the mouse and brood size were kept in the models for total provisioning, conditional provisioning and total care.

Table 4.1: Differences in parental and offspring behaviours based on varying larval demand, manipulated through the age of the brood in the burying beetle *Nicrophorus vespilloides*. I present the parameter estimates (and standard errors) for each behaviour trait, which measure the difference between the treatment in question and the control treatment within a time point, in comparison to the difference between the two in the first observation. Level of statistical significance of each effect is indicated by stars (. $<$ 0.1, * $<$ 0.05, ** $<$ 0.01, *** $<$ 0.001). Estimates are derived from generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors. Estimates for additional covariates presented in footnote (a, b, c). (See Appendix A for Z-scores and p-values.)

		Behaviour trait				
	Factor	Total provisioning	Begging	Conditional provisioning	Total care	Larval interactions
Treatment	Young	0.20(1.43)	0.38(1.35)	0.30(1.45)	0.38(1.25)	0.73(1.91)
	Middle-aged	-0.53(1.46)	-0.36(1.38)	-0.43(1.46)	0.73(1.27)*	2.41(1.83)**
	Old	-1.49(1.53)**	-1.83(1.43)***	-1.43(1.54)**	0.72(1.28)*	2.82(1.82)***
Timepoint	Time(25)	-0.07(1.46)	0.42(1.35)	0.01(1.47)	-0.01(1.24)	1.18(1.94)
	Time(49)	-0.74(1.51)	-0.78(1.37)*	-0.68(1.51)	0.14(1.25)	1.21(1.93)
	Time(73)	-2.75(1.87)***	-2.16(1.60)***	-2.21(1.87)**	0.18(1.28)	0.81(2.05)
Interaction	Young:Time(25)	0.35(1.56)	0.03(1.46)	0.25(1.57)	-0.10(1.31)	-1.21(2.21)
	Middle-aged:Time(25)	-0.70(1.77)	-0.74(1.62)	-0.44(1.75)	-0.69(1.35)*	-2.78(2.22)**
	Old:Time(25)	-0.27(1.87)	-0.53(1.65)	-0.32(1.84)	-0.70(1.36)*	-2.55(2.13)**
	Young:Time(49)	0.68(1.62)	1.08(1.48)*	0.61(1.62)	-0.49(1.33)	-1.51(2.25)
	Middle-aged:Time(49)	1.27(1.69)*	1.14(1.53)*	1.17(1.68)*	-0.54(1.36)	-2.22(2.14)*
	Old:Time(49)	0.90(1.83)	0.70(1.61)	0.62(1.81)	-0.39(1.35)	-2.73(2.11)**
	Young:Time(73)	2.58(2.09)**	2.98(1.80)***	2.25(2.08)*	-1.03(1.49)*	-1.23(2.45)
	Middle-aged:Time(73)	2.98(2.07)***	2.12(1.78)**	2.43(2.07)**	-0.86(1.41)*	-2.46(2.36)*
	Old:Time(73)	2.68(2.19)**	2.03(1.82)**	1.97(2.16)*	-0.65(1.38)*	-1.78(2.18)*

(a) Mouse mass [0.90(1.05)*], and Brood size [1.36(1.09)***]
(b) Mouse mass [0.91(1.05).], and Brood size [1.32(1.09)**]
(c) Mouse mass [0.94(1.04).], and Brood size [1.12(1.05)*]

4.3 Results

4.3.1 *Total provisioning*

Overall, the pattern of total provisioning was different in the control treatment than in the three experimental treatments (Figure 4.2). In the young treatment, the pattern of total provisioning resembled that of the control, but the larvae were provisioned for more at the end of the experiment (interaction terms with young in Table 4.1, Figure 4.2a). The higher levels of provisioning at this stage suggest that females adjust their behaviour to the age of the larvae to accommodate the higher needs of young larvae. Provisioning for the mid-aged larvae fluctuated over time (interaction terms with mid-aged in Table 4.1, Figure 4.2a). The levels of provisioning remained at the same level throughout the experiment in the old treatment, with the larvae being fed less than the larvae in the control treatment overall (main effect of old treatment in Table 4.1, Figure 4.2a). Thus, there is no support for either extremes of complete larval or parental control of resource allocation. Finally, provisioning also declined with increasing mouse mass, and increased with increasing brood size (Table 4.1).

4.3.2 *Begging and parental responsiveness*

Overall, the pattern of begging in all treatments corresponded well to prediction of the larval control over food allocation (Figure 4.1b), as the larvae adjusted their behaviour on cues about their own condition rather than following cues received from the parent (Figure 4.2b). While the control treatment followed the expected pattern based on previous studies (Smiseth et al., 2003, Figure 4.2b) the amount of begging increased throughout the experimental period in the young treatment (interaction terms with young in Table 4.1, Figure 4.2b). The pattern of begging in the mid-aged treatment also differed from the pattern of the control treatment (interaction terms with mid-aged in Table 4.1), with begging staying at the same level throughout (Figure 4.1b). The old larvae begged less overall throughout the experiment, and the level of begging was approximately constant throughout (main effect of treatment and the interaction terms with old in Table 4.1, Figure 4.2b). The overall pattern of

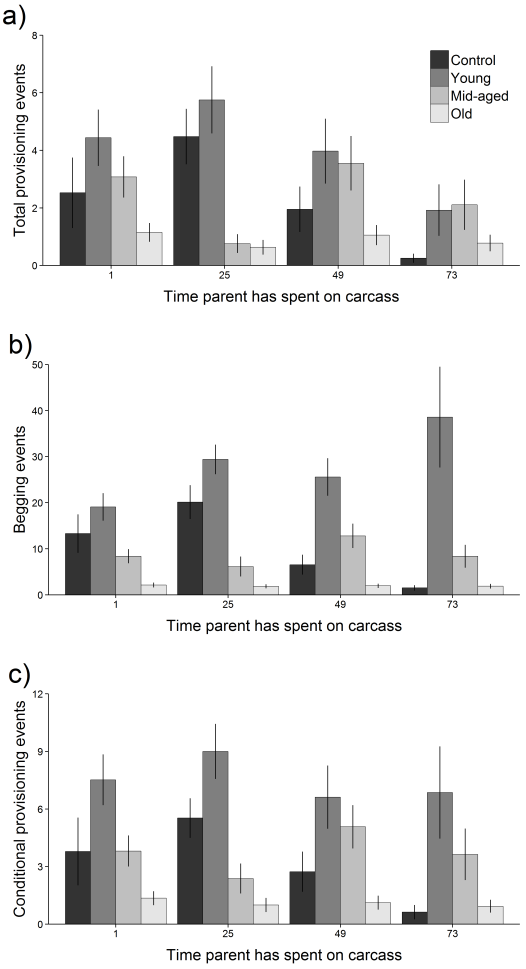


Figure 4.2: Mean (+/- standard error) of behaviour traits related to resource allocation, observed during each 30 minute behaviour observation conducted in 24 hour interval. (a) Count of total provisioning events during the observation. (b) Count of the number of larvae begging during the observation. (c) Count of provisioning events when the female was within a pronotum length's distance from the larvae (conditional provisioning).

conditional provisioning (provisioning when the parent was within a pronotum length's distance of the larvae) closely resembled the pattern of begging with each treatment producing similar patterns as observed for begging (Table 4.1, Figure 4.2c). Hence, when the parents were within distance of the larvae, their responsiveness to begging remained the same throughout the experiment in all treatments, suggesting offspring control over parental care in these traits.

4.3.3 *Total amount of care*

The overall pattern of total amount of care was different in the control treatment and the three experimental treatments, showing that other parental care behaviours changed as a consequence of the observed changes in total provisioning (Figure 4.2a). In the control treatment, females provided the same amount of care at all stages of juvenile development (Table 4.1, Figure 4.3).

When larval demand declined as the larvae grew older, females tended to switch to indirect forms of care (i.e. maintaining the carcass or guarding the larvae), but still spend the same amount of time caring for the brood in total. The mismatching of the expectations of the female and the need of the larvae led to changes in the patterns of total care (Table 4.1, Figure 4.3). In the young treatment, the total amount of care declined over time (interaction terms with young in Table 4.1, Figure 4.3). Larvae in the mid-aged and old treatments received more care overall (main effects of treatment in Table 4.1), and the levels of care fluctuated over time (interaction terms with mid-aged or old in Table 4.1, Figure 4.3). Total care declined with increasing mouse mass, and increased with increasing brood size (Table 4.1). These changes in the pattern of total amount of care provided reflect changes in parental behaviours as a whole: A decrease in total care implies an increase in nonparental behaviours. I also analysed the pattern of all behaviour traits separately (Appendix B), and that the amount of time the female spent interacting with the larvae showed an unexpected peak in two of the mismatched treatments. The females spent very little time interacting with the larvae overall, except for the first observation of the mid-aged and young treatments (Table 4.1, Appendix B). I observed the parents grooming the larvae on 46 occasions in the mid-aged, and 95 occasions in the old treatment, when similar observations were only made 10 times in the control, and 3 times in the young treatment. The peak in larval interactions seems to derive from parental attempts to provide care, and may suggest that the female was assessing the larvae in the beginning of the experiment.

4.4 Discussion

I found that *N. vespilloides* females provisioned at higher levels at the end of the experiment in all the mismatched (i.e. experimental) treatments in comparison to the matched one (i.e. control), suggesting that females adjusted their provisioning based on cues they received from the age (and thus, the need) of the brood. The realised levels of provisioning did not, however, fully correspond to the predictions of either full parental or full offspring control, suggesting that control over parental care is a compromise between the parents and the offspring. The theoretical models for the resolution of parent-offspring conflict can be divided into two classes: scramble competition (Parker et al.,

2002a), and honest signalling models (Godfray, 1991, 1995a,b). Scramble competition models (Parker et al., 2002a) assume that offspring have behavioural control, and that the parental response to begging is evolutionarily fixed. Honest signaling models assume that parents have behavioural control, as they actively allocate resources to the offspring according to the honest signals indicated by begging (Godfray, 1991, 1995a,b). In reality, the realised control over resource allocation may often lie between these two extremes, with the power shifting from parents to offspring and back throughout offspring development (Royle et al., 2002). Below, I provide a discussion of the mechanisms of controlling parental care, for both the offspring and the parents, to explain the observed differences in the level of food provisioning between the matched and mismatched treatments.

If the offspring were fully in control, I would expect to see the larvae begging based on their own need (i.e. age), and the parents adjusting their behaviour fully to the need of the larvae (Figure 4.2b). Furthermore, I might expect the larvae to increase their level of begging when paired with parents who are expecting high levels of begging as a means to manipulate the parent into providing more care than they would otherwise be willing to provide (Parker and Macnair, 1979; Trivers, 1974). As expected, I found that the larvae based their begging on cues about their own age, rather than adjusting their

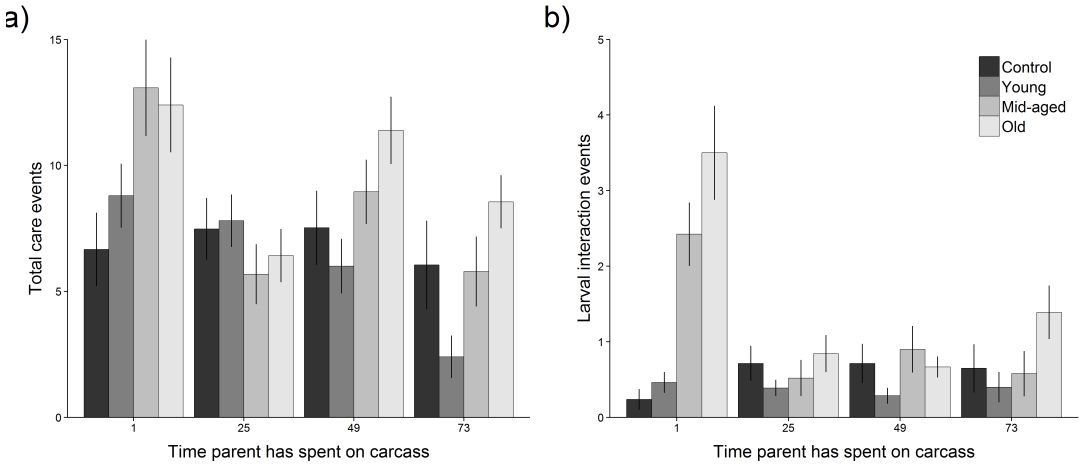


Figure 4.3: Mean (+/- standard error) of behaviour traits observed during each 30 minute behaviour observation conducted in 24 hour interval. (a) Count of total caring events, encompassing both feeding the offspring and providing indirect care for them. (b) Count of occasions where a parent was interacting with the larvae without feeding it, encompassing behaviours such as grooming the larvae.

behaviour to the expectations of the female. However, there was no evidence for the larvae manipulating the parents by begging at higher levels when paired with a parent who was expecting more demanding larvae than the actual age of the brood (Figure 4.2b). My findings are in accordance with previous studies on the species. Changes in begging behaviour of *N. vespilloides* larvae have been shown to reflect their hunger state (Smiseth and Moore, 2004a, 2007), suggesting that begging is an honest signal of need (Smiseth and Moore, 2004a). It has also been shown that while larvae are capable of discriminating between certain classes of adults (Chapter 2), they do not behave differently towards adults from different stages of development (Leigh and Smiseth, 2012), which is consistent with the lack of exaggeration in my findings. As such, my data on begging is in accordance with the honest signalling models (Godfray, 1991, 1995a,b). Thus, given that the larvae beg based on their own age, the observed differences in parental provisioning were not due to manipulation by the larvae. Conversely, in a previous experiment with a similar setting on Manx shearwaters, the chicks exaggerated their begging by begging more in the mismatched setting, regardless of their body condition (Riou et al., 2012). A tendency for manipulation was also found in earwigs (*Forticula auricularia*), though it was dependent on the age of the offspring, as young nymphs reacted to cues about maternal condition by attempting to exploit the parents when possible, but old nymphs showed no reaction (Wong et al., 2014).

The parent can take control over parental care through two mechanisms: becoming less responsive to begging over time, or by adjusting the amount of time spent near the larvae. The first mechanism would allow them to avoid provisioning more than they would expect the larvae to need at any given time. In that case, I would expect to see the direct response to begging (i.e. conditional provisioning) to follow the pattern of the control treatment in all treatments. My findings show a different pattern, as the females were consistent in how they responded to begging throughout the experiment, thus provisioning based on actual larval age (Figure 4.2c). Considering the honesty of the begging signal, this finding is not that surprising. According to honest signalling models, when begging is a true indication offspring need, the parent optimises its fitness by responding to it (Godfray, 1995b). In contradiction to my findings, Riou et al. (2012) found that the responsiveness of Manx shearwater parents dropped at the later stages of development regardless of the begging exhibited by the chicks. This discrepancy between different studies may be due to differences in

the future reproductive potential of burying beetle and shearwater parents. Parental investment into the current brood is expected to decrease when parents have a high potential for future reproduction (Stearns, 1992; Ward et al., 2009; Wong et al., 2013), the same expectations may also apply to the responsiveness of the parent. For example, parents of the New Zealand passerine, the hihi *Notiomystis cincta*, respond to changes in their potential to reproduce again by becoming less sensitive to the begging of their current offspring (Thorogood et al., 2011). However, not much is known about the likelihood of multiple reproductive bouts for *n. vespilloides*, but they are severely limited by competition for carcasses used in breeding (Eggert and Müller, 1997; Scott, 1998). Thus the value of the current reproductive attempt may be higher to the beetles than it is to the shearwaters in the study by Riou et al. (2012), or to the hihi's in the study by Thorogood et al. (2011).

As parents did not change their responsiveness to begging throughout the experiment, we might expect the parents to adjust the amount of time they spend on behaviours pertaining to parental care, either to compensate for the resources put into provisioning, or to regulate the amount of time spent near the larvae. For instance, a female caring for a brood that keeps begging at high levels regardless of the amount of provisioning it has already received from the female may choose to abandon the brood, move away from the larvae, or spend more time consuming carrion to regain some of her lost energy reserves. Thus, the female may counteract the offspring's attempts to control the amount of parental care she provides by removing herself from the presence of the larvae. I found that the total amount of care given by females in the control treatment stayed at the same level during all observations. In the experimental treatments, however, females provided more care to the brood when caring for mid-aged and old larvae, and the amount of care declined over time when caring for young larvae. This pattern may be explained by the perceived differences in the quality or value of the offspring. In the European earwig (*Forticula auricularia*), mothers who had been exposed to chemical cues of either broods with high food availability or low food availability, showed more care when they perceived their brood to be of high quality, and more aggression when they perceived their brood to be of low quality (Mas and Kölliker, 2011). The older larvae of the mid-aged and old treatments may thus be assessed as being of good quality, potentially due to their size or associated traits, therefore prompting the female into overcompensating the amount of care given. My finding that the females in

the mid-aged and old treatments appeared to assess the broods suggests that the females are looking for cues of offspring need and quality. Parents exhibit similar assessment on the size of the carcass, which influences their decisions to cull their larvae (Müller and Eggert, 1990). Thus, my finding suggests that the parents do adjust the total amount of time spent caring when their expectations are mismatched with the actual age of the larvae.

In conclusion, I find that the level of parental resource allocation is mediated through cues received from offspring begging, but adjusted through parental behaviours. The parents compromise over the amount of care given by remaining responsive to larval begging, but regulate the interactions by changing the amount of time spent on other behaviours. My finding contrasts previous studies finding that the parents have an active role in determining the within brood resource allocation (Andrews and Smiseth, 2013; Smiseth and Morgan, 2009). However, the previous studies manipulated the asynchrony of the broods (Andrews and Smiseth, 2013; Smiseth and Morgan, 2009), and thus they also included asymmetric sibling competition, which is absent in my experiment where all larvae in the broods were of the same competitive rank. Therefore, it is possible that sibling competition makes the larvae regulate themselves, allowing parents to take control more easily. The presence of parents in itself drives sibling competition, as asynchronous broods without parents do not suffer similar costs as asynchronous broods with parents (Smiseth and Moore, 2007). Sibling competition normally follows the patterns predicted by scramble competition models (Parker et al., 2002b), as in the beetles, the older larvae are generally either more competitive or favoured by the parents (Smiseth and Parker, 2008). Competition among offspring is as such also an important factor in determining the control over parental care (Mock and Parker, 1997).

4.4.1 *Concluding remarks*

My results provide empirical evidence on the control over the level and duration of parental care being a compromise between the parents and the offspring. Most previous studies have found support for either parents (eg. Kilner and Johnstone, 1997; Kölliker et al., 1998; Rauter and Moore, 1999; Riou et al., 2012; Smiseth and Morgan, 2009 or offspring (Parker et al., 2002a; Rodríguez-Gironés et al., 2001; Smiseth et al., 2003) having control over these

traits. The dichotomy in assessing the question of control arises from the assumptions of honest signalling (Godfray, 1991, 1995a,b) and scramble competition models (Parker et al., 2002b), as the former assumes parental control, and the latter assumes offspring control. In reality, the assumptions of these two models lie at the opposite ends of a continuum, with the power shifting between the parents and the offspring based on the circumstances and offspring development (Royle et al., 2002). Furthermore, the experimental designs used in previous work addressing the same question, have been too simplistic to detect a compromise between parents and offspring, thus skewing the results towards either end of the continuum. With my cross-fostering design that was conducted throughout offspring development, I was able to detect both the parents and the offspring having influence over the control over parental care, which would have remained undetected in the more simple experimental designs of previous work. Thus I conclude that future work in this field needs to be based on more complex experimental designs that take into account the possibility of a shifting power continuum, and that acknowledge that the mechanisms of both models may not be mutually exclusive. My findings provide empirical evidence for the idea that control is shared between parents and offspring, with each party having some influence over the other.

Chapter 5

Offspring demand affects parental life history trade-offs

Abstract

Life-history trade-offs for the number and size of offspring produced, and the reproductive costs in future reproduction and survival can all be affected by different levels of parental effort. Because of these trade-offs the parents and the offspring have different optima for the amount of care given to the current brood, which leads into a conflict between parents and offspring. The offspring, as well as the parents, have the ability to affect parental effort, and thus changes in offspring traits have the potential to cause reproductive costs on the parents. I used a repeated cross-fostering design to manipulate offspring demand during juvenile development in the burying beetle *Nicrophorus vespilloides* to examine whether responding to offspring begging incurs reproductive costs on the parent. After a manipulated first reproductive event, I gave each experimental female, that had been exposed to different levels of larval begging, a change to breed again, and monitored their survival. I found that an increase in larval demand changes the realisation of the trade-off between number and size of the offspring, whereas a reduction does not. I also found that the parents paid a fecundity cost for the general success of their first broods, but did not show costs on other traits, including survival. Survival and the number of larvae successfully raised in the second broods correlated positively, indicating differences in the individual quality of the parents. This is the first time reproductive costs induced by larval traits other than their number, have been reported.

5.1 Introduction

Maximising parental fitness during multiple reproductive events requires successful optimisation of resource allocation between current and future reproduction, as well as survival (Stearns, 1992). Within broods, the parents face a trade-off between the number and size of offspring produced (Lloyd, 1987; Smith and Fretwell, 1974), and between broods, they must balance out the reproductive costs (as first proposed by (Williams, 1966) that caring for the

current brood incurs for their future reproduction (eg. Gustafsson and Sutherland, 1988; Monaghan and Nager, 1997), or survival (eg. Dijkstra et al., 1990). Previous work investigating costs of reproduction have included observational studies on correlations between life-history traits (eg. Bryant, 1979), and experimental studies that have manipulated parental effort through traits related to current reproduction (such as clutch size, eg. Hodges et al., 2015; Kölliker et al., 2015), or by adding to the energetic costs of care for the parents (such as handicapping the parent with weights; eg. Harding et al., 2009; Hegemann et al., 2013; Tieleman et al., 2008). The evidence for these life-history trade-offs still remains controversial, as many empirical studies have not found the expected trade-off between the life-history traits, or have even found a positive relationship instead of the expected negative correlation (reviewed in Reznick, 1985; Roff and Fairbairn, 2007). The lack of phenotypic trade-off in some studies can be due to quality differences between the individuals used in the study (ie. among-individual heterogeneity, (Weladji et al., 2008, but see Wilson and Nussey, 2010), or for example unaccounted for sex differences (see Santos and Nakagawa, 2012). However, trade-offs discovered through inducing different levels of parental effort have so far ignored the potential for parental effort to change based on the traits and the behaviours of the offspring themselves, potential for which has been reported widely in other contexts (Agrawal et al., 2001; Kilner and Johnstone, 1997; Parker et al., 2002b; Rodríguez-Gironés et al., 2001; Smiseth et al., 2003). Aside from manipulations of offspring number, no studies have been conducted to test the effects of manipulating the offspring's behaviour rather than the costs and benefits of care to the parent. Furthermore, offspring influence may significantly change the dynamics behind life-history trade-offs, and even account for some of the variation caused by the unknown factor, often assumed to be due to individual quality, that can mask the assumed underlying life-history trade-offs.

Offspring influence over parental care relies on behavioural plasticity of the parent in responding to the cues received from the offspring, as offspring demand has an effect on parental supply (Hussell, 1988). Offspring induced plasticity has been reported before: for example, Kight (1997) found that the duration of care and parental defence was affected by cues from the offspring, which were eventually overridden by the parent's internal clock in the burrower bug *Sehirus cinctus*. Theoretically this plasticity is beneficial for the parent only up to a point where the benefits from a unit of care given are higher than

benefits from investing the same unit into future reproduction. Furthermore, the gains from future reproduction change based on parent's likelihood to breed again. Indeed, (Thorogood et al., 2011) found that the parents changed their responsiveness to offspring food solicitation signals when their potential to breed again was manipulated through food supplementation, in a New Zealand passerine, *Notiomystis cincta*. Therefore, as the parents are trying to optimise their care across multiple broods, and the offspring benefit from each unit of investment they receive, the optimal levels of care are different for the parents and offspring, which leads to a conflict between the parents and the offspring (Trivers, 1974). Parent-offspring conflict has the potential to sway the trade-offs between parental effort and life-history traits to a direction determined not only by the resource allocation constraints affecting the parent, but also by the need of the offspring. Both the parents and the offspring can bias the amount of parental investment towards their own optimum through influencing one another through parent-offspring communication (reviewed in Kilner and Johnstone, 1997). This communication has in many taxa evolved into elaborate begging displays used by the offspring to elicit food from the parents, and to give the parent honest information about the condition of the offspring (Kilner and Johnstone, 1997). Parental care is not the only factor determining the condition of the offspring: their phenotypic condition is also largely affected by environment (eg. Merilä, 1996). Therefore, to optimise the amount of care given to the offspring, the parents not only need to assess their potential to breed again, but also assess the potential pay-off they can receive from their current offspring based on their current condition.

In this study, I experimentally manipulated offspring demand during the first reproductive event to investigate whether offspring demand can incur a reproductive cost to the parent. The burying beetle, *Nicrophorus vespilloides*, is an excellent system for such experiments, as the larvae of the species exhibit begging behaviour, the intensiveness of which is highly determined by larval age, and as such can be manipulated with ease (Smiseth et al., 2003). Burying beetles feed and breed on carrion of small vertebrates and provide post-hatching parental care for the offspring (Eggert and Müller, 1997; Scott, 1998). Usually only the females stay with the brood until the larvae disperse from the carcass into the soil to pupate (Scott, 1998). The larvae are capable of feeding from a suitably prepared carcass on their own (Eggert et al., 1998), but their growth and survival are positively affected by parental food provisioning (Smiseth and

Moore, 2004a). Larvae beg by touching the adults with their feet (Smiseth and Moore, 2002; Smiseth et al., 2003), and the parents respond by provisioning pre-digested carrion to their broods (Scott, 1998). As the larvae become more proficient in self-feeding during juvenile period, the intensity of begging in a brood declines, leading to age-dependent variation in the amount of begging exhibited by an individual larva (Smiseth et al., 2003). Begging peaks at 24 hours after hatching, and starts declining after that, until approximately 72 hours after hatching, which marks the point of transitioning to nutritional independence (Smiseth et al., 2003). Here, I manipulated the age of the brood female burying beetles were caring for during their first reproductive event, in order to manipulate the level of offspring demand the females experienced. My aim was to test whether the female parents would exhibit cumulative reproductive costs associated with changes in parental effort in the current reproductive event, by responding to the manipulated demand from the larvae. I predict that changes in larval demand would lead to changes at different stages in the life-history of the parent, namely in (1) the size and number of offspring produced, (2) the success of the second brood, and (3) the survival of the parent.

5.2 Materials and methods

5.2.1 *Origin and husbandry of the beetles*

The beetles used in this experiment derived from a large, outbred laboratory population originating from wild-caught beetles trapped in Corstophine Hill and Craiglockhart Hill (Edinburgh, UK), Kennall Vale, (Cornwall, UK) and Madingley Woods (Cambridge, UK). All experimental beetles that were housed individually at different stages of the experiments were housed in the same conditions (for further details of the housing conditions, see Chapter 2).

5.2.2 *Experimental treatments: first broods*

I randomly selected pairs of nonsibling virgin male and female beetles to be mated. All matings were conducted in transparent containers (12 x 18 x 6 cm)

filled with 2 cm of moist soil and a previously frozen mouse carcass to breed on (range 20-25 g, supplied by Livefoods Direct Ltd, Sheffield, UK). I only used female parents in this experiment to remove the confounding effects of sex-differences in the expression of the life-history trade-offs, and chose females because male care is highly variable and has no detectable effects on larval growth or survival under laboratory conditions (Eggert et al., 1998; Smiseth et al., 2005). Thus, I removed the male 60 hours after pairing, which is before the larvae started hatching. Concurrently, in order to have control over the age of the experimental broods and prevent any contact between the female and the larvae prior to brood age manipulations, I moved the female and the carcass into a new container filled with soil to separate the eggs from the breeding female. The egg boxes were checked 5 times a day for hatching. When the larvae started hatching, I assigned some females into treatments, and used others to create donor broods for the brood age manipulations. The donor females were assigned mixed maternity broods of 15-25 newly hatched larvae from the supply of broods that had started hatching in the egg boxes. These donor broods were then used to create experimental broods after they had reached an age appropriate for the treatment of the experimental female they were given to.

I manipulated larval demand in the experimental broods by repeated cross-fostering for the approximate duration of larval dependency (i.e. first 72 hours after hatching). Larval begging behaviour changes during larval development, peaking at 24 hours after hatching, and declining after that as the larvae become more proficient in self-feeding (Smiseth et al., 2003). I gave each experimental female a brood of 10 mixed maternity larvae of a known age, and swapped the brood with another experimental brood of a known age systematically throughout the experiment, using a supply of donor broods consisting of larvae of an appropriate age. I generated four treatments that differed with respect to the ages of the broods. (1) The broods of the control females were initially set up with newly hatched larvae, and the broods were always swapped with a brood consisting of larvae of the same age as the ones taken away. This control group was created to control for the effects of handling on larval and parental behaviours. (2) To create a treatment with high larval demand (hereafter referred to as the high demand treatment), I kept the larvae young throughout the manipulation period, by supplying the female with a brood of 1-hour-old larvae, which were always swapped as they reached the age of 25 hours to another brood of 1-hour-old larvae. (3) In the intermediate

demand treatment, the female was given 25-hour-old larvae, which were swapped at the age of 49 hours with another brood of 25-hour-old larvae. (4) Finally, in the low demand treatment, the female was given 49-hour-old larvae, which were swapped at the age of 73 hours with another brood of 49-hour-old larvae. In all treatments, the larvae were swapped four times in total during the manipulation period. To capture the initial behaviour of the females when given larvae of a different age than expected (Chapter 4), the first swaps were conducted an hour after the initial broods were given to the females. To ensure that the age of these first broods corresponded to the rest of the experimental manipulations, the first broods given to the females consisted of newly hatched, 24-hour-old, 48-hour-old, and 72-hour-old larvae in the control, high, intermediate, and low demand treatments, respectively. An hour later these broods were swapped, and the swaps were then continued as determined previously. After the last swap, the female was allowed to raise the larvae until they dispersed from the carcass.

When the brood had dispersed, i.e. when all larvae had moved from the carcass to the soil around it, the female was removed. I then weighed the broods to the nearest 0.1 mg using a digital scale (Ohaus Pioneer, with 0.1 mg accuracy), and counted the number of surviving larvae. To account for any cumulative treatment effects on the survival of the offspring, I kept the broods in their containers (12 x 18 x 6 cm) and monitored their survival through pupation, until they eclosed as adults. However, the differences in survival across all treatments were not big enough to give me the statistical power needed for testing these effects at my sample sizes, leading me to only use the data descriptively. Due to mortality in the donor broods, experimental females were occasionally discarded in the middle of the experiment, as there were no larvae to provide for them. Thus, my final sample sizes for the control treatment, and the high, intermediate, and low demand treatments were 20, 27, 19 and 18, respectively.

5.2.3 *Recording reproductive costs: second broods and lifespan*

To record reproductive costs of caring for broods with different levels of larval demand, I mated the experimental females for a second time to an unrelated

virgin male, 3-11 days after the dispersal of the first broods. The males were again removed 60 hours after hatching to remove the confounding effects of male care. I then monitored the unmanipulated second broods to determine the reproductive success of the females. To assess fecundity, I counted the eggs laid at the bottom of the containers, visible through the transparent plastic, which correlates strongly with the total number of eggs laid (Monteith et al., 2012). I checked the boxes daily first for hatching, and then for dispersal, as well as the unlikely death of the female or the brood. At dispersal, I weighed the broods and counted the number of larvae, to assess reproductive success in the number and size of larvae that the females successfully raised. After the larvae had dispersed, I moved the females to individual housing in smaller containers. The boxes were checked daily for the death of the females. The recorded day of death was then used to determine the lifespan of an experimental female in days, starting from the day when it was first mated. I measured the length of the female's pronotum after her death with a Mitutoyo absolute digimatic caliper (1-150 mm). Four females in the high demand treatment died before they could be mated again, and three beetles escaped their containers during mortality tracking, leaving our final sample size on survival data with 19, 26, 19 and 17 for the control, high, intermediate and low treatments, respectively.

5.2.4 *Statistical analyses*

All analyses were conducted with R version 3.1.2 (R Core Team 2014). I used linear mixed effects models (package lme4, Bates et al., 2014) as all response factors (individual larva mass at dispersal, number of eggs, number of larvae at dispersal, and lifespan) had a gaussian error distribution. In all analyses, I included experimental block as a random variable. First, I explored the relationship between the number and size of offspring within different treatments in both first and second broods, separately. I assigned the mean (individual) larva mass at dispersal for the respective broods being investigated, as the response variable. Treatment (control, high, intermediate and low), brood size at dispersal were then assigned as fixed factors, as well as two-way interactions between the factors. Secondly, I explored the brood size at dispersal in a similar manner, by assigning the brood size for the respective broods as the response variable. Treatment (control, high, intermediate and low) and mean size of larvae at dispersal were again assigned as fixed factors, as well as

two-way interactions between the two. For the analyses on larval size and number in the second broods, I added the size and number of the first broods as covariates, to assess whether any detectable effects were affected by the success of the first reproduction.

Next, I analysed the costs of reproduction in three separate models for the three traits I predicted them to be found in: number of eggs laid, number of larvae at dispersal, and lifespan of the female. In the analyses on the number of eggs and the number of larvae at dispersal, I assigned treatment and the number of larvae at dispersal of the first broods and the two-way interaction between the two as fixed factors. For the analyses on the number of larvae at dispersal (for the second broods), I added the number of eggs, and the two-way interactions between it and the other fixed factors (treatment: number of eggs, number of larvae at dispersal of the first broods: number of eggs) to the model, as the traits in question might influence one another. Similarly, in the analysis on the lifespan of the females, I also added the number of larvae at dispersal for the second broods, and the two-way interactions between it and the other fixed factors (treatment: number of larvae at dispersal of the second broods, number of eggs: number of larvae at dispersal of the second broods, number of larvae at dispersal of first broods: number of larvae at dispersal of the second broods).

I also assigned mouse masses of the first and second reproductive events as covariates in all models, as the amount of resources available affects the number and size of offspring produced (Smiseth and Moore, 2002). To decrease the number of variables in the analysis on lifespan, I counted a total lifetime mass of resources available for reproduction by adding the sum of the two mouse masses into the model as a covariate, instead of the two separate masses. I also wanted to account for parental state, as size and age of the parent are strong determinants for traits associated with the success of offspring produced (Kindsvater and Otto, 2014). Therefore, I assigned female age and size as covariates in all the models. To attain parsimonious models, non-significant ($P<0.1$) terms were removed based on ANOVA's comparing the maximum likelihood estimates of the nested models. My data had a disproportionate number of young females in the different treatments. Thus, when female age had a significant effect in the case of analyses about the costs of reproduction, I repeated the analyses with a subset of the data only using the females that were older than 20 days at the time of the first reproduction to test for the

Table 5.1: Factors affecting the size of offspring in two reproductive events of the burying beetle *Nicrophorus vespilloides*. All estimates are derived from a linear mixed effects model (lmer), with degrees of freedom and *P*-values attained through Satterwaithe approximation. The number of the brood is indicated in brackets after each variable that was measured in both reproductive events (1= first broods, 2= second broods).

Response	Factor	Par	SE	df	t-value	*P*-value	
Larvae size (1)	Treatment						
	High	-0.02	0.01	72	-2.61	0.011	*
	Intermediate	-0.01	0.01	71	-2.04	0.045	*
	Low	-0.01	0.01	71	-1.71	0.091	.
	Brood size (1)	0.01	1.65×10^{-3}	73	3.29	0.002	**
Brood size (1)	Treatment						
	High	-0.23	0.06	9	-3.85	<0.001	***
	Intermediate	-0.07	0.06	-4	-1.18	0.241	
	Low	0.67×10^{-3}	0.05	-1	0.01	0.990	
	Larvae size (1)	-0.23	0.06	3	-3.85	<0.001	***
Larvae size (2)	Treatment						
	High	-0.03	0.02	56	-1.77	0.082	.
	Intermediate	0.02	0.02	57	1.11	0.270	
	Low	0.00	0.02	60	0.05	0.964	
	Brood size (2)	-1.66×10^{-3}	0.58×10^{-3}	61	-2.83	0.006	**
	Mouse mass (2)	0.01	2.27×10^{-3}	60	2.29	0.026	*
	Treatment: Brood size (2)						
	High: Brood size (2)	1.49×10^{-3}	0.72×10^{-3}	58	2.07	0.043	*
	Intermediate: Brood size (2)	-6.69×10^{-4}	0.88×10^{-3}	58	-0.76	0.449	
	Low: Brood size (2)	9.88×10^{-5}	0.92×10^{-3}	60	0.11	0.914	
Brood size (2)	Treatment						
	High	0.15	0.15	2	1.82	0.073	.
	Intermediate	0.13	0.13	2	1.99	0.051	.
	Low	0.13	0.13	2	1.71	0.092	.
	Larvae size (2)	1.77	1.77	-4	-3.63	0.001	***
	Larvae size (1)	2.14	2.14	1	0.91	0.369	
	Female age	-7.96×10^{-3}	2.75×10^{-3}	-3	-2.90	0.005	**
	Mouse mass (2)	0.04	0.04	2	1.82	0.073	.

robustness of the results. In this occasion, my sample sizes were 17, 16, 13 and 16, for the control, high, intermediate and low treatments, respectively. As no differences from the overall main results were found, only the results of the original models with full data are reported.

5.3 Results

5.3.1 *Number and size of offspring*

The experimental manipulations limited the number of larvae in the first broods to a maximum of 10 larvae, which was less than the number of larvae produced in the unmanipulated second broods (Figure 5.1a). Generally, the size of the larvae in the first broods increased with increasing brood size (Figure 5.1c, Table 5.1), and with decreasing larval demand (see treatment order in Figure

5.1b, Table 5.1).

The unmanipulated second broods consisted of more larvae (Figure 5.1a), that were on average more closely matched in size in the different treatments than the larvae of the first broods (Figure 5.1b). In the second broods, the relationship between the number and size of offspring was non-linear, with an increase in larval size up to brood sizes of approximately 20 larvae, and a decrease after that (Figure 5.1c). The increase in brood sizes for the second broods, in general, had a negative effect on the size of the offspring produced (Figure 5.1). Furthermore, the detected differences in larval sizes and numbers in the second broods were driven by differences in the number of larvae surviving to dispersal, as apparent by the significant interaction term between treatment and brood size (Table 5.1). Larval number, however, was dependent on the experimental treatment directly, which was due to the high demand treatment differing from the pattern of the control treatment (Table 5.1). The females in the high demand treatment produced fewer offspring than the control females in the first brood and more in the second brood (Figure 5.1a), but the size of the offspring increased towards the second brood unlike in the control treatment, where the size decreased (Figure 5.1b). Although the second broods of the intermediate and low demand treatments appeared to have more offspring (Figure 5.1a), these treatments followed the pattern of the control treatment, when the noise caused by other factors was corrected for (Table 5.1). The size of the carcass also had a positive effect on the size of the offspring (Table 5.1).

Table 5.2: Factors affecting the life-history traits likely to show reproductive costs in female burying beetles (*Nicrophorus vespilloides*). All estimates are derived from a linear mixed effects models (lmer), with degrees of freedom and P-values attained through Satterwaithe approximation. The number of the brood is indicated in brackets after each variable that was measured in both reproductive events (1= first broods, 2= second broods).

Response	Factor	Par	SE	df	t-value	*P*-value	
Number of eggs (2)	Larvae number (1)	-2.06	0.86	67	-2.40	0.019	*
	Female size	4.67	6.86	67	0.68	0.498	
	Mouse mass (1)	2.04	1.17	67	1.74	0.086	.
Number of larvae at dispersal (2)	Larvae number (1)	0.87	0.45	59	1.93	0.059	.
	Egg number (2)	0.61	0.07	58	8.50	<0.001	***
	Female size	-1.52	3.52	59	-0.43	0.667	
Lifespan	Larvae number (1)	-0.96	0.92	59	-1.05	0.300	
	Larvae number (2)	0.61	0.18	59	3.31	0.002	**
	Female size	9.39	7.47	59	1.26	0.214	
	Female age	-0.25	0.12	59	-2.15	0.036	*

5.3.2 *Reproductive costs*

The number of eggs produced in the second broods of the experimental females declined significantly with each surviving offspring produced in the first broods, which was corrected for by the size of the carcass in the first broods, as well as the size of the female (Figure 5.2a, Table 5.2). Thus, the parents paid a fecundity cost for the general success of their first broods (Figure 5.2a). Considering only the high demand treatment differed significantly in the number of larvae produced (Table 5.1, Figure 5.1a), the fecundity cost is mostly paid by the females in the high demand treatment, where parental effort was - in theory - increased, rather than reduced (Figure 5.2a). The number of larvae dispersing from the second broods was correlated with the number of eggs produced in the second broods, although the number of larvae dispersing from the first broods also had a near significant positive effect as well (Table 5.2, Figure 5.2b). All in all, there were no signs of a reproductive cost paid at the level of number of larvae dispersing from the second broods. The number of larvae in the second broods in turn positively affected the lifespan of the females (Table 5.2), indicating that the females producing larger second broods also had a longer lifespan (Figure 5.2c).

5.4 Discussion

The main aim of this study was to explore whether offspring begging has an influence over parental life-history trade-offs and traits associated with them. I found that the experimentally manipulated levels of larval demand had some effect on the trade-offs between number and size of offspring produced (Table 5.1), but did not directly incur a reproductive cost to the female parent (Table 5.2). However, I found that increased larval demand influenced the number of offspring surviving from the first broods, which in turn influenced the future fecundity of the female (Table 5.2, Figure 5.2a). Therefore, an increase in larval demand induced an indirect reproductive cost to the females, while decreased larval demand had no impact on any traits associated with reproductive costs. My results resemble those found on a meta-analysis on birds, where costs paid in survival were only apparent when parental effort was increased, while decreased parental effort had no effect on the future success of the parent (Santos and

Nakagawa, 2012). This is the first time offspring behaviour has been reported to have an impact to life-history trade-offs of the parent, in a similar manner to parental traits (Bryant, 1979; Harding et al., 2009; Hegemann et al., 2013; Tieleman et al., 2008) or manipulations of offspring number (Hodges et al., 2015; Kölliker et al., 2015). Here, I will discuss these findings in more detail.

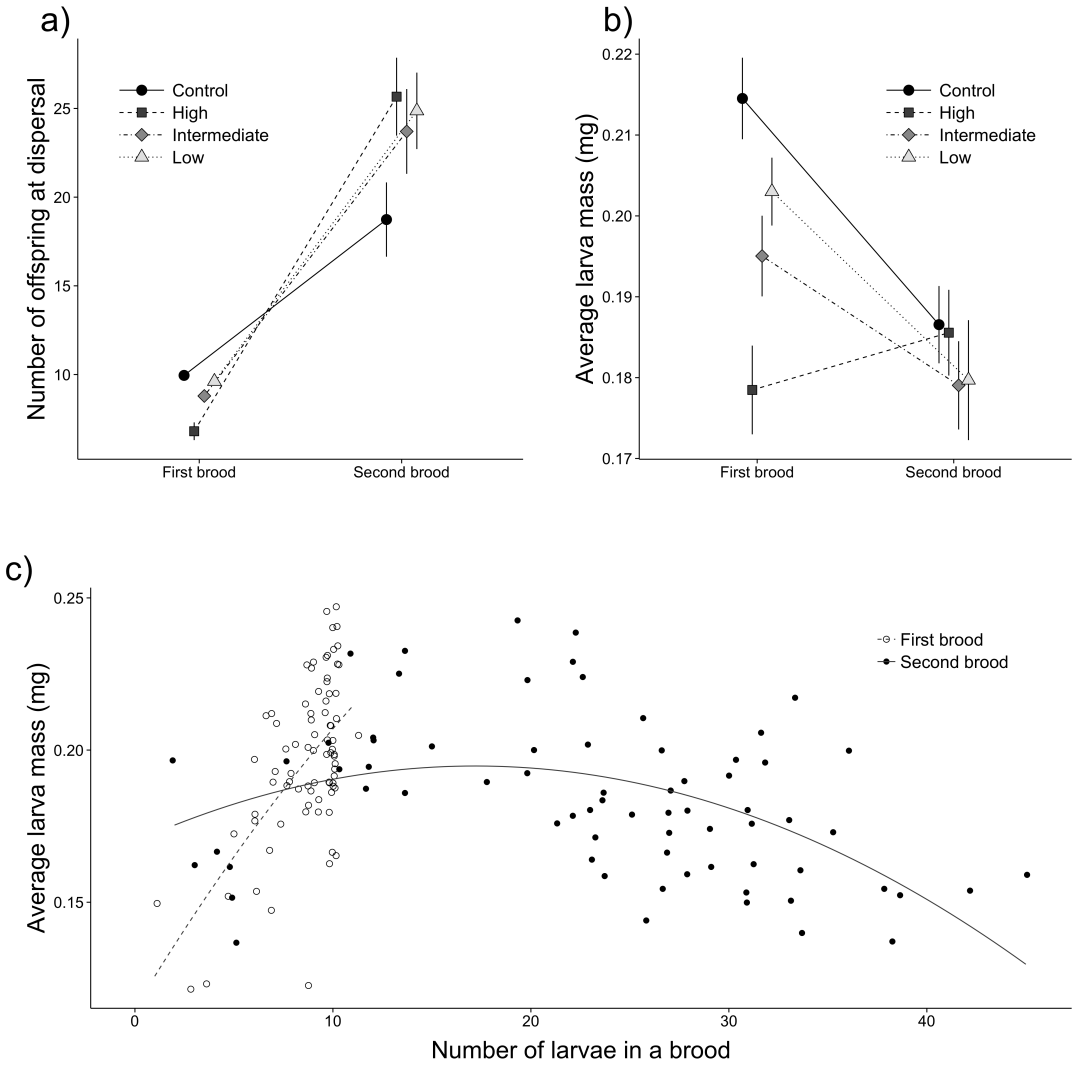


Figure 5.1: Size and number of offspring in manipulated first broods, and the unmanipulated second broods of the female burying beetles (*Nicrophorus vespilloides*). The number of larvae was kept at a maximum of 10 larvae that were not related to the females in the first broods, while it was allowed to vary freely in the biological second broods. The treatments indicate different levels of manipulated larval demand (control, high, intermediate and low). (a) Number of offspring (mean +/- SE) dispersed from the first and second broods. (b) Individual body mass (mean +/- SE) at dispersal for the larvae produced in the first and second reproductive events. (c) Individual body mass (brood means) at dispersal in relation to brood size.

My results show that in *N. vespilloides*, the success of the first brood incurs a reproductive cost in fecundity, but not in the number of larvae dispersing from future broods, or in the survival of the parent. Similar effects on fecundity induced by manipulations of parental effort have been implied in previous work: Parejo and Danchin (2006) found that blue tits (*Parus caeruleus*) were less likely to undertake a second reproduction when they had been rearing enlarged broods, but found no evidence for other reproductive costs. Golet et al. (2004) found that black legged kittiwakes (*Rissa tridactyla*) were more likely to breed next year if their eggs were removed the year before, finding that the fecundity costs, when they appeared, were cumulative and appeared in multiple traits. Kölliker et al. (2015) found that the genetic trade-offs between current and future reproduction were more severe before hatching than after hatching. All of these studies imply a difference between the expression of reproductive costs in pre-hatching traits, such as fecundity, and other traits, with the other traits sometimes following a similar pattern to the initial costs apparent at the pre-hatching stage (Golet et al., 2004; Kölliker et al., 2015), and sometimes showing no other costs at all (Parejo and Danchin, 2006). Reproductive costs on fecundity in terms of the number of zygotes produced have been studied less than the number of recruiting offspring, partly due to the majority of data being generated on cavity nesting birds, for which counting eggs imposes bigger methodological difficulties than monitoring recruits. Therefore, data on fecundity costs in the number of zygotes produced, is still lacking, and their

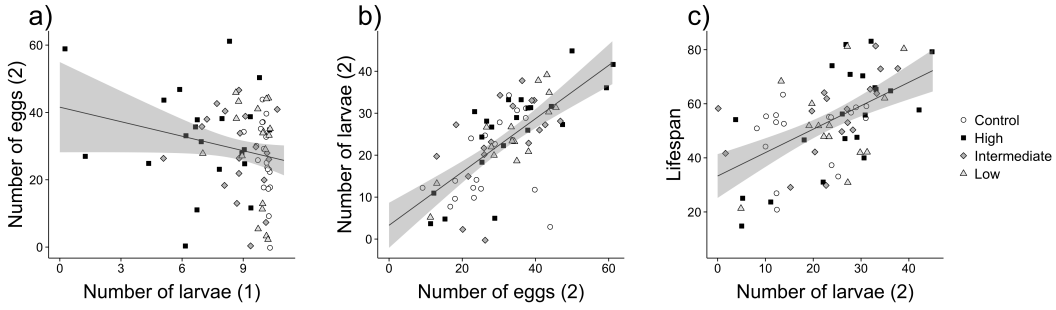


Figure 5.2: The strongest relationships explaining variation in each life-history traits. (a) The relationship between the relative success of the first broods (number of larvae dispersing) and the number of eggs laid in the second broods. The negative relationship indicates a reproductive cost in fecundity. (b) The relationship between number of eggs in the second broods and the number of larvae dispersing from the same broods. (c) The relationship between number of larvae dispersing from the second broods and the lifespan of the females. The number of the brood is indicated in brackets after each variable that was measured in both reproductive events (1= first broods, 2= second broods). The grey areas indicate standard errors for the regression lines.

existence may well be more common than currently reported data suggests. These initial production costs may very well show the reproductive costs more readily, as they are not under the influence of parental behaviours or conflated by the fitness of the second generation.

In my data, I found a strong correlation between the number of eggs laid and the number of larvae dispersing from the second broods, but only the number of eggs laid was affected by the success of the first broods. The disparity between the two results may be due to parental care masking the costs at the post-hatching stage of juvenile development. In *N. vespilloides*, a similar effect has previously been reported on the effects of egg size on larval size at dispersal: Monteith et al. (2012) found that the size of the larvae at dispersal was determined by the size of the eggs in broods that were raised without parents, but not in ones with them. Thus, it is possible that parental care can also buffer against the post-hatching reproductive costs. Alternatively, the lack of an apparent reproductive cost in the number of larvae at dispersal, can also be tied to the self-feeding ability of *N. vespilloides* larvae (Eggert et al., 1998). The ultimate success of the larvae is a combination of both parental care and their own ability, and the two may also be linked: Providing care for the burying beetle larvae may also improve the larval ability to self-feed. To investigate the effects of larval success by combining both their own ability, and parental input, and thus accounting for the link between the two traits, we need to consider both the number of offspring (which can be affected by the parent directly) and the size of the offspring (which is a combination of parental effort and larval self-feeding). My results show that when larval size was not taken into account, the number of larvae dispersing from the second broods was not determined by the previous reproductive success or the treatment. However, when larval size was taken into account (in the analyses on the size of offspring at dispersal, Table 5.1), the number of larvae dispersing from the second broods differs significantly from the control treatment in the treatment where offspring demand was at its highest (Figure 5.1a). Thus, there is some indication that the parents do pay a reproductive cost in the number of larvae dispersing as well, though this is only apparent when the relative size of the larvae is also taken into account. The difference between the results on larval size and larval number may be caused simply by differences in larval quality, or by differences in larval condition caused by parental effort that improves both larval condition and larval self-feeding. As I cannot tease the two effects apart in my

experiment, it is important for us to consider the results of both situations.

When both larval size and number are taken into account, I found that the females of the high demand treatment produced more offspring in their second broods that were also larger than those of their first broods, while the rest of the experimental females produced more offspring of a smaller size than the offspring of their first broods (Figure 5.1a,b). There are three potential explanations for the increase in larval size in second broods of the high demand treatment. Firstly, the elevated need to provision the larvae during the first reproductive event, may have forced the females to increase their consumption of the carcass for their own maintenance, at the expense of decreasing the available resources for the offspring in the first brood. The additional resources gained by the female may even have left them in a better condition than the control females, with more resources to spend on producing the second broods. However, the deaths in the high demand treatment (4 observed deaths against no deaths in other treatments) prior to the second reproductive event, suggest that the females were on the contrary in a worse condition at the end of the first reproduction than the control females. Secondly, the females in the high demand treatment may have had a skewed perception of the reward gained from the parental investment allocated to the first brood, as the females increased their provisioning (Chapter 4), but ultimately in their first broods, produced offspring that were fewer and smaller than the control broods. Therefore the parent may have been tricked into investing more into the second broods as well. However, should this have been the case, I would have expected the other treatments to show the pattern for the opposite, as the low demand in the first reproductive event should have then induced a lower investment into the second broods. Previous studies on *N. vespilloides* have also found that prior experience has no effect on the mating tactics used by the adult beetles (Walling et al., 2008), and as such it is unlikely it would affect reproductive tactics either. Thirdly, the expended effort in raising the first brood may have triggered the females into adapting terminal investment strategy (Clutton-Brock, 1984; Creighton et al., 2009). Stressors may induce the likelihood of terminal investment: as an example food limitation in the yellow mealworm beetles (*Tenebrio molitor*) increased terminal investment into reproduction (Krams et al., 2015). High demand from the larvae may as such have been a signal of a stressful environment for the females in my experiment.

I found differences in the number and size of offspring produced in the high demand treatment, but not in the other treatments. The difference between this treatment and the intermediate and low demand treatments, is that the level of parental effort is expected to increase rather than reduce in comparison to the natural setting (control treatment). Thus, my result resembles that of a meta-analysis on birds, which reported that reproductive costs on survival tended to only be expressed when the levels of parental effort were increased rather than reduced (Santos and Nakagawa, 2012). Positive effects of an energy surplus in treatments where parental effort is reduced from the normal setting, are potentially less plausible, as the females are already likely to be allocating an optimal amount of resources into their current reproduction. Therefore the resources saved from current reproduction may well be invested in other traits, such as those improving the likelihood of gaining another mating opportunity, rather than increasing the number or size of offspring produced during the second reproduction. Male birds have been found to increase their attractiveness when parental effort in previous reproductive event was reduced (Gustafsson et al., 1995; Siefferman and Hill, 2005). Parents of titmice have also been shown to transfer some of their reproductive costs onto their offspring in order to maintain their own condition (Linden and Mø ller, 1989). While my data cannot differentiate between these options, the offspring of the first broods died before adulthood more often in the high demand treatment than in the other treatments (N(all larvae dead before eclosion in the first broods) = 1,6,0,2 for the control, high, intermediate and low treatments, respectively). Thus, there is tentative evidence for the poorer quality of the offspring in the high demand treatment, which could potentially indicate that parents are transferring some of their costs onto the offspring. However, conclusive determination of where the excess resources are used in *N. vespilloides* requires further experimental work.

I found a positive relationship between the number of larvae produced in the second broods and the lifespan of the females (Figure 5.2c). Previous studies investigating the life-history trade-offs have often reported positive relationships between parental effort and life-history traits, rather than the expected negative trade-offs (Reznick, 1985; Roff and Fairbairn, 2007). These positive relationships have often been considered to be a result of differences in individual quality within the study population, as the good quality individuals invest more into a variety of life-history traits connected to fitness and low quality individuals invest less to the same traits (Roff and Fairbairn, 2007).

These differences in individual quality can thus mask the underlying trade-off, if there is one (see Wilson and Nussey, 2010). In their meta-analysis on birds, Santos and Nakagawa (2012) found that there were differences in the expression of survival effects based on the sex of the parent. Females were found to be as likely to survive as control females when parental effort was increased, whereas the males expressed costs of reproduction through survival (Santos and Nakagawa, 2012). Therefore, as I only used females in this study, it is possible that while the burying beetle females did not suffer from survival costs, the males potentially might have. My results from experimental manipulations on offspring demand therefore show the same ambiguity that previous research exploring the relationship between parental effort and survival have found. Thus, offspring traits, though having a role in determining the outcome of resource allocation and incurring other types of reproductive costs, do not affect the reproductive costs paid on survival. Survival is thus still likely to be determined by differences in individual quality.

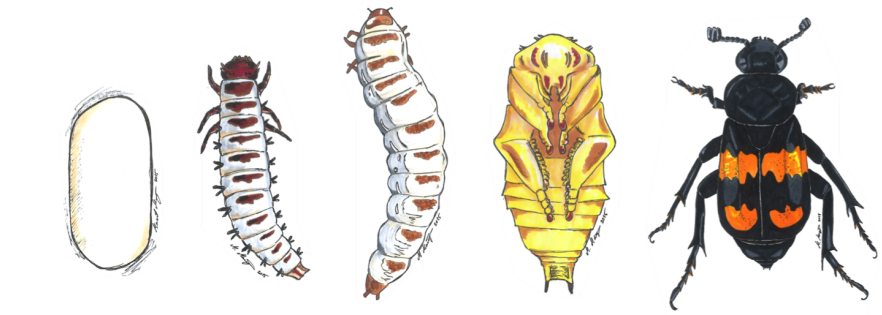
Lastly, I also found that the overall shape of relationship between the number and size of offspring was non-linear, with an increase at the brood sizes below 20 larvae, after which the average mass of an individual larva in a brood steadily decreases as the brood size increases (Figure 5.1c). The same trend is found when examining only the shape of the trade-off curve for the second broods, whereas the manipulated first broods, which only consisted of a maximum of 10 larvae, showed a linear increase in average larva mass over the number of larvae in a brood (Figure 5.1c). Previous work on *N. vespilloides* and other species of the same genus have reported the classic negative, linear trade-off curves between brood size and the size of the larvae, with the mass of the offspring decreasing with increasing brood size (Smiseth et al., 2014; Trumbo, 1990). Resource availability has been shown to have an influence on the shape of the trade-off between number and size of offspring in *Nicrophorus* species before, as the linear relationship has either been shown to be steeper on smaller carcasses (Bartlett and Ashworth, 1988; Scott and Traniello, 1990; Smiseth et al., 2014), or to only affect the number of larvae produced, rather than their size as predicted by the classic life-history theory (Bartlett and Ashworth, 1988; Smith and Fretwell, 1974; Trumbo, 1990; Wilson and Fudge, 1984). The trade-offs have also been shown to only be apparent at the dispersing larvae stage, rather than at the egg production stage, meaning that parental care seems to drive the existence of this trade-off (Monteith et al.,

2012). Virgin beetles tend to produce approximately 0.8 - 1.4 offspring per a gram of resource on their first reproductive event (reported for *Nicrophorus tomentosus*; Trumbo, 1990, and a similar relationship observed for *N. vespilloides*; personal observation M.I. Mäenpää). Because of this, it can be assumed that the broods are only limited by resources after a certain number of offspring have been produced, the treshold for which is dependent on the size of the carcass. Therefore, we can assume that the broods that are smaller than the optimal size based on the size of carcass available, are not limited by resources, and thus there can be an increase in both the number and size of offspring produced up to the point when the carcass size becomes limiting. After that, the trade-off between number and size can operate.

In conclusion, I found that an increase in offspring demand had an effect in the life-history trade-offs of the parent. While there was a fecundity cost of the general success of the first broods, the females were able to buffer against this effect at later stages through parental care, without influencing their future survival. As parents that produced more offspring also survived better, the individual quality of the parent plays a role in the resolution of these life-history trade-offs. Offspring demand did not provide me with a potential explanation for the existence of the positive correlations between life-history traits that are expected to be traded off against one another, and instead produced a similar pattern. Individual quality of the female is thus presumably a more important trait in resolving resource allocation between and within broods than offspring demand. However, as offspring demand does have an impact on fecundity, it is nevertheless important to consider its role in changing the nuances behind life-history trade-offs when they do occur.

Chapter 6

General discussion



6.1 The mechanistic basis of begging, and its social consequences

I present evidence that the begging behaviour of *Nicrophorus vespilloides* larvae is adjusted based on parental cues, and can directly affect parental behaviours, as well as incurring a reproductive cost to the caring parent in terms of their future fecundity. Parent-offspring communication is widely regarded as having evolved to provide the parent with honest information about the hunger state of the offspring, which is supported both in many theoretical models as well as empirical studies (Godfray, 1995a,b; Kilner and Johnstone, 1997; Parker and Macnair, 1979; Parker et al., 2002a; Wright and Leonard, 2002). The behaviour is seen as a mediator for the conflict over resource allocation between parents and offspring (Godfray, 1995a). Parent-offspring conflict arises because the offspring benefit from receiving more care than the parents are selected to provide (Trivers, 1974). I studied both the mechanistic basis of begging behaviour, and its consequences to the caring parent, to determine what cues the offspring use in adjusting their begging behaviour, and how the parent responds to unexpected changes in the levels of begging.

I found that the larvae did not discriminate between the male and female beetles, but did adjust their care to different classes of adult beetles, including responding differently towards familiar and unfamiliar beetles, indicating individual recognition of the adults (Chapter 2). The levels of begging were always high towards breeding adults regardless of whether they had been previously exposed to parental cues or not, which suggests that the offspring have an innate template for responding to the chemical cues from the parent (Chapter 2). I also found no evidence that egg size and thus the initial size of the offspring affected the amount of begging, indicating that either the offspring do not use cues of their long-term needs when begging, or that egg size does not represent these cues well (Chapter 3). These results in combination provide more insights into the mechanisms behind adjusting begging behaviour. I also found that changes in the levels of begging cause both an immediate behaviour response from the caring parent (Chapter 4), and that this response incurs a

reproductive cost in fecundity for the parent (Chapter 5). My findings indicate that the parents are willing to adjust their behaviour to the need of the offspring, even at a cost to their later life-history. Other studies have also found that the offspring have the ability to influence parental resource allocation to future reproduction (Hinde et al., 2010; Meunier and Kölliker, 2012), but no studies before have demonstrated a reproductive cost caused by begging behaviour. Here I will discuss the wider implications of my findings, and the new directions that they suggest to the research of parent-offspring communication and conflict.

6.2 Mechanistic basis of begging

In Chapter 2 and Chapter 3, I explored different aspects of the mechanistic basis of begging in order to determine what cues *N. vespilloides* offspring use to adjust the amount of begging they exhibit. When investigating the reliability of begging signals, it is important to understand the mechanistic basis of begging behaviour, as otherwise it would be possible to overlook any possible physiological limitations to what the offspring are capable of detecting. I explored whether chemical cues from different types of adult beetles trigger begging (Chapter 2), and whether initial offspring size, as one of the potential innate cues determining offspring long-term need, would have an effect on the level of begging exhibited by the larvae (Chapter 3). A wide range of literature has shown that offspring of many species adjust their begging to their hunger state (reviewed in Kilner and Johnstone, 1997; Mock et al., 2011; Wright and Leonard, 2002), but to what degree other factors, such as learning or offspring condition, are involved in determining the amount of begging is still uncertain (but see Kedar et al., 2000; Price et al., 1996). Knowledge of what determines the amount of begging exhibited by the offspring can also provide more information about the whether begging behaviour can be used plastically based on changes in the cues available to the offspring. The reliability of begging may depend on these cues, as the offspring may exhibit different levels of begging in different situations to attain the maximal resource allocation (Kedar et al., 2000), and the information contained in the absolute level of begging exhibited may be different to different recipients. Responding to wrong cues is costly (e.g. Budden and Wright, 2001; Leonard and Horn, 2001; Leonard et al., 1997), and

it is these costs that maintain the honesty of begging signals, and advocate the need for discrimination for cues that are used to adjust begging behaviour.

6.2.1 *Begging based on chemical cues from the parent*

The burying beetle larvae showed no discrimination between the male and female parents, but they did beg more towards breeding than non-breeding adults, and even showed discrimination between unfamiliar beetles than familiar beetles (Chapter 2). Interestingly, this individual recognition did not indicate that the larvae favoured the adult beetles that were caring for them, as they actually begged more towards unfamiliar caring beetles, contrary to results attained in other studies (e.g. Aubin and Jouventin, 1998). As begging increases the risk of being cannibalised by the parent (Andrews and Smiseth, 2013), for such a response to have evolved, it would be expected that the either the benefits received from begging towards unfamiliar beetles would be higher than the benefits received from begging towards a familiar beetle, or that the costs of begging are lower in the case of the unfamiliar beetles. In general, the energetic costs of begging are very low or non-existent in the burying beetles (Smiseth and Parker, 2008). However, it is unknown whether the risk of being cannibalised by an unfamiliar beetle would be lower than the risk posed by a familiar parent. From the offspring perspective, a fresh parent may represent potential additional resources arriving from outside their own carcass, and therefore it may be plausible that the benefits received from unfamiliar beetles are higher from the offspring point of view. However, as the unfamiliar parent is likely to be unrelated to the begging offspring, it would also be logical for it to respond to begging of unfamiliar larvae more aggressively than it would respond to begging of its own offspring. Therefore, other factors may be in place explaining the higher levels of begging towards unfamiliar adults, one of them potentially involving a type of learning by the offspring.

My results, in general, show no evidence for offspring learning: the discrimination exhibited by the larvae required no prior exposure to the parent, suggesting it was based on an innate template (i.e. difference between breeding and non-breeding beetles). However, as the offspring can recognise their own parent, it is possible that they have optimised the level of begging to fit the responsiveness of the familiar parent. When a new caring adult arrives, the

offspring would best ensure that they receive the maximum rewards from a new parent by starting to beg at higher levels than they normally do, in order to learn the threshold level of responsiveness of the newcomer. Therefore, the higher response to new caring adults may be due to offspring learning to beg at different intensities towards different individuals. Differences in parental responsiveness to begging can in some species teach the offspring to beg at different levels based on the reward they receive from their parent (Kedar et al., 2000). However, the likelihood of encountering the same unfamiliar beetle repeatedly in the wild may be relatively low, thus making the mechanism of learning rather unlikely. However, some species of the same genus, including *N. vespilloides*, do occasionally breed communally, with multiple pairs of beetles raising their offspring on the same carcass (Komdeur et al., 2013; Scott, 1994; Trumbo, 1992; Trumbo and Fiore, 1994). This communal living provides the beetles with enhanced ability to use large carcassess (Trumbo and Fiore, 1994), and to compete against flies attempting to use the same resource (Scott, 1994), even if the relative benefits are rather low in comparison to costs associated with communal living (Komdeur et al., 2013). In communal living, the offspring are likely to encounter multiple caring adults repeatedly, and thus offspring might be able to learn to beg at different levels towards any potential carers. My data cannot, however, determine the cause of high levels of begging towards unfamiliar caring adults, and further studies are needed to examine why the larvae respond differently towards these unfamiliar beetles than towards the familiar beetles.

6.2.2 *Begging based on long-term needs of the offspring*

I found that the egg size of the burying beetles correlates with multiple fitness traits of the offspring, but there is no relationship between begging and offspring size (Chapter 3). My results indicate that either the offspring do not use cues of their long-term needs derived from their initial size, or that their long-term needs are not associated with egg size in the first place. However, I cannot rule out the possibility that the offspring of *N. vespilloides* beg based on long-term needs that are determined by traits other than egg size (such as hatching order or body condition). While size often correlates with fitness, even in *N. vespilloides* (Otronen, 1988; Steiger, 2013), on its own it may not be a good indicator of offspring condition, and may therefore be a poor indicator of

offspring need. Taking into account other traits, may, however, change the relative importance of egg size: Egg composition, for example, has been linked to laying order of the eggs in species of fish and birds (Fuiman and Ojanguren, 2011; Gilby et al., 2012; Rice et al., 2013), and it is possible that differences in initial size of the offspring would have a different impact at different stages of the laying sequence, as it would also be associated with changes in egg composition. In my study, all larvae that were used to create experimental broods were quite likely to be among the first eggs that hatched, due to the experimental design. Therefore I cannot completely rule out the possibility of a relationship between egg size and begging, but if it exists, it is likely that it would be driven by covariation with other relevant traits.

My results show that egg size correlated with offspring quality, as large egg size was associated with both faster growth, larger attained body size, and improved survival (Chapter 3). By definition, long-term needs are determined as the growth rate that an offspring must attain to reach a target mass by the end of the juvenile period (Price et al., 1996). Egg size, having an impact on growth rate, should therefore have the potential to determine the long-term needs of the larvae. However, this does not seem to be the case. What may cause the lack of a relationship may ultimately be due to a potential difference in two closely related terms used to address individual's state, *quality* and *condition*. Current literature rarely makes a difference between the two terms, and in most cases the two are used as synonyms. While a point has been made about the lack of definition to quality in many studies being a problem (Wilson and Nussey, 2010), no studies have yet made a definite distinction between quality and condition. While I will not attempt to give an official definition for the two terms, the two may have very different implications: Intuitively quality would indicate the potential fitness that an individual can aspire to achieve due to its physiological, or genetic limitations, while condition might indicate short term fluctuations in the realisation of this fitness. Good quality individuals may, for example, be better at recovering from a bad condition than bad quality individuals would be. If this differentiation between the two terms would be true, size is likely to reflect offspring quality, but not necessarily condition. Long-term needs of the offspring, on the otherhand, are more likely to be based on condition, as condition can be changed more easily than quality. A lack of definite definition for the two terms in literature, however, makes predicting these potential differences difficult, and indicates a clear gap in knowledge that

needs to be addressed.

6.3 Consequences of begging on current and future parental behaviour

In Chapter 4 and Chapter 5 I explored the consequences that responding to begging imposes on the caring parent. I investigated both the immediate behavioural response of the parent during the approximate time of dependency in the juvenile period (Chapter 4), and the potential reproductive costs of these different responses (Chapter 5). Offspring adjust their begging on the cues received from the parent (Leigh and Smiseth, 2012, Chapter 2), but to what degree the parents base their behaviour on offspring signals or their own state is largely unknown. Knowing the degree to which either parents or the offspring can influence one another improves our understanding of what drives parent-offspring conflict, and which theoretical model is the best representation of the real world. In addition, it is important to know how flexible begging behaviour is, and whether there are changes in its reliability over time. Reproductive costs induced by responding to begging would also change our understanding of how begging evolves, as its effects on life-history trade-offs have otherwise been understudied.

6.3.1 *Effects of begging on the current parental behaviour*

I found that offspring of *N. vespilloides* based their begging on their own state, rather than taking cues from the caring parent, when the age of the offspring and the reproductive state of the parent were mismatched over the approximate period of dependency during the caring period (Chapter 4). This finding is consistent with a previous finding that the offspring do not change their begging behaviour based on cues about which stage of reproduction the parent originates from (Leigh and Smiseth, 2012), and my results also show that this effect does not change as offspring age. The parents, however, provisioned food according to offspring's begging behaviour, although they did show signs of reducing the amount of begging over time when it was excessive (Chapter 4). This indicates

that the parents are willing to provision more food than they would expect necessary based on how much time has passed since their larvae had appeared on the carcass. Therefore, the offspring can influence the amount of provisioning given by the parent, but the parent does also reduce the amount of provisioning it gives over time. My results show that the resolution of parent-offspring conflict does not simply give control to either the offspring or the parent, or follow one theoretical model, but rather the resolution of the conflict is a compromise. Thus, my results fit in with previous work suggesting that the two extremes of parental control or larval control (represented by different models) form the two ends of a continuum, where the control over parental care shifts from the parents to the offspring in different situations (Royle et al., 2002).

My results show that the parents that received offspring that were far older, and thus far bigger, than their own larvae would be, spent more time grooming the larvae, as well as providing more indirect care for them, than the parents of younger larvae did for their broods (Figure 4.3). Partially this is likely to be due to the older larvae exhibiting less begging, thus giving the parents more time to engage in behaviours other than food provisioning. However, the parents of older larvae actually ended up spending more time providing care, when both indirect and direct forms of care were taken into account (Figure 4.3a). One possibility for this is that the parents were using the size cues derived from the offspring to assess the quality (determined for this purpose as a combination of traits correlated positively with fitness) of the offspring, to assess the marginal fitness benefits they receive from caring for them. Should this be the case, it is possible that the parents are allocating different types or levels of care based on offspring need than offspring quality. Other studies have shown that the parents change their responsiveness to begging based on their own potential to breed again (Thorogood et al., 2011), showing a behavioural change based on a change in the cost and benefit ratio of producing a future brood. Therefore, it would not be too unlikely to assume that there could be a behavioural change based on an increase in the benefits of caring for the current brood as well. However, I cannot tease apart the potential cues of offspring size (or age) from the different levels of begging exhibited by different-aged larvae, and therefore further studies are needed to evaluate whether begging itself plays a part in determining the value of the broods for the parent.

6.3.2 *Reproductive cost on the parent caused by offspring begging*

I found that elevated levels of offspring begging during the first reproductive event incurred a reproductive cost to the parent in its future fecundity, but not in other traits (Chapter 5). The costliness of parental care, in general, is well documented (Clutton-Brock, 1991; Royle et al., 2012). Long-term parental influence on the offspring has also been shown in previous studies: For example, the long-term consequences of parental care have been shown to have a profound impact on fitness-related traits of the offspring as they reach adulthood, including their lifespan (Bateson et al., 2004; Nussey et al., 2007). Recently, Kilner et al. (2015) showed that the levels of care received as a juvenile also carry over to the offspring's ability to raise broods of its own later in life, as the offspring that received little care became low quality parents. However, as my results show, the offspring can also influence the parent in long-term through begging behaviour (Chapter 5), and offspring have also been shown to have an effect on the resource allocation between broods (Hinde et al., 2010; Meunier and Kölliker, 2012). Therefore, there are severe consequences of parental care to both the life history of the offspring, and of the parent. The threshold value to avoiding providing care is therefore likely to be relatively high, allowing some variation in the expression of need from the offspring.

Offspring influence over parental trade-off between current and future reproduction (i.e. reproductive cost on future fecundity) also indicates a need to include information about life-history trade-offs into the models examining the resolution of parent-offspring conflict. Costs and benefits of begging to both the offspring, and to the parent are more complex than most theoretical models assume. Therefore, ignoring the long-term costs and benefits apparent only when examining the life history consequences to both the parent (Chapter 5) and the offspring (Bateson et al., 2004; Kilner et al., 2015; Nussey et al., 2007), may have contributed in part to the difficulty in finding empirical support for many assumptions of the theoretical models, including the costs of begging (Wright and Leonard, 2002). The life-history consequences of begging and parental care may provide a framework for determining when the different theoretical models would be favoured against one another, as the fluctuations of costs and benefits are likely to have an affect on parental decisions to care. However, further research is needed to test the importance of these associations.

6.4 The importance of begging earnest

Honest begging is important in the theoretical models investigating parent-offspring conflict, as when begging is not a reliable signal of need, the parent would ultimately evolve to ignore begging rather than respond to it (Godfray, 1995a,b; Parker and Macnair, 1979). I found no evidence of the offspring manipulating the parents, even when the offspring were cared for by parents that were expecting larvae to beg at higher levels than the offspring's own age indicated (Chapter 4). Therefore offspring signaling was reliable towards their own parents. Whether the reliability of the signal is maintained when begging towards other caring adults, however, is undetermined: One possible explanation for my finding of the high levels of begging towards unfamiliar beetles (Chapter 2) could potentially be that the offspring exaggerate their needs to manipulate an unfamiliar caring adult. However, my results do not allow me to differentiate this explanation from other potential explanations. Furthermore, the likelihood of encountering unfamiliar caring adults in the wild is very low, and as such, the chances of evolving a response such as manipulation in an otherwise honest signaling system, are also extremely low, making the explanation very unlikely.

Parents may also use cues about offspring state (be it a more easily determined state such as size, or age, or a more ambiguous term such as quality, or condition), which may be expressed through means other than begging. Some indication to this is found in Chapter 4, where the parents spent more time interacting with old larvae. Additionally, Kight (1997) found that up to a certain point in the caring period, the parents of the burrower bug *Sehirus cinctus* followed age cues received from their young to determine the amount of defence they provided. Therefore, to attain full understanding of parent-offspring communication, it may be necessary to investigate other cues that accompany begging, as they may change parental responsiveness to begging. Honesty of begging is still likely to be important even when cues about offspring state are taken into account. Short-term needs of the offspring, including their hunger levels, fluctuate and as such the honesty of begging signals ensures the satiation of these needs similarly for offspring of any state. Any potential additional information about the state of the offspring would be likely to influence parental decisions to respond (Riou et al., 2012; Thorogood et al., 2011), but it might also affect parental ability to detect exaggerated

signaling, which in turn would maintain the honesty of the signal. Yet, it is possible that the marginal benefits received from food provisioning may differ for different offspring, as offspring may be able to use the additional resources better at different states. The benefits received from forms of care other than food provisioning may also be higher to some offspring than the benefits of meals provided by the parents in species where the offspring can survive without parental care. Work addressing size hierarchies due to hatching asynchrony (e.g. Mainwaring et al., 2014; Smiseth and Morgan, 2009; Smiseth et al., 2006) address some of these issues. However, more work is required to determine whether parents use other cues in combination with the information attained through begging signals in other situations.

6.5 Concluding remarks

Here I have shown further evidence of the complex nature of offspring begging and parental responsiveness to it, and how it is adjusted based on different cues. My results show that the resolution of parent-offspring conflict is a compromise between parent and the offspring (Chapter 4), supporting the view of the overlapping nature of different theoretical models (Royle et al., 2002). Chapter 2 shows that offspring beg discriminatively between different classes of adult beetles, and have the ability to recognise individual adults, and to adjust their begging accordingly. Interpretation of the results of Chapter 3 give some indication that there is a need for a clear definition for the difference between the terms *condition* and *quality*. Both can be used as the potential long-term needs examined as a part of the theoretical models for the resolution of parent-offspring conflict, but their meaning is likely to be different. Re-examining the terminology and its application to empirical work used to address offspring long-term needs is therefore needed in the future. Chapter 4 shows that the assumptions of either the honest signaling models proposed by Godfray (1995a,b) or the scramble compettition models (Parker and Macnair, 1979) are fully met, and thus neither of them perfectly explain the outcome of parent-offspring conflict. Chapter 5 shows that parents do pay a price for responding to begging in terms of their future fecundity, thus showing that life-history trade-offs might influence the resolution of parent-offspring conflict. Based on this evidence, it is important to consider the complex nature of

begging behaviour in studies addressing parent-offspring conflict, as many factors affect how it is adjusted, and its influence over parental traits can be profound.

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Appendix A.

Supplementary material for Chapter 4

A table for full model results of behaviour traits analysed in Chapter 4

Table A.1. Summary of statistical tests for behaviour traits of the burying beetle *Nicrophorus vespilloides*. For each factor on the treatment level, there is information on parameter estimate (Par), standard error (SE), test statistic (Z value), P value (P), effect size (ES), and the standard error of the effect size (SE(ES)). All behaviour traits were analysed with generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors.

	Factor	Par	SE	Z value	P	ES	SE(ES)
Total provisioning	Young	0.19	0.36	0.54	0.587	1.22	1.43
	Mid-aged	-0.54	0.37	-1.44	0.151	0.59	1.45
	Old	-1.49	0.43	-3.50	< 0.001	0.23	1.53
	Time(25)	-0.08	0.38	-0.22	0.826	0.92	1.46
	Time(49)	-0.74	0.41	-1.81	0.070	0.48	1.51
	Time(73)	-2.76	0.62	-4.42	< 0.001	0.06	1.87
	Brood size	-0.10	0.05	-2.06	0.039	0.90	1.05
	Carcass mass	0.31	0.09	3.48	0.001	1.36	1.09
	Young:Time(25)	0.34	0.44	0.78	0.438	1.41	1.56
	Mid-aged:Time(25)	-0.70	0.57	-1.22	0.223	0.50	1.77
	Old:Time(25)	-0.26	0.62	-0.42	0.672	0.77	1.86
	Young:Time(49)	0.67	0.48	1.40	0.162	1.96	1.62
	Mid-aged:Time(49)	1.24	0.52	2.39	0.017	3.46	1.68
	Old:Time(49)	0.91	0.60	1.50	0.133	2.47	1.83
	Young:Time(73)	2.65	0.74	3.59	< 0.001	14.15	2.09
	Mid-aged:Time(73)	2.96	0.72	4.09	< 0.001	19.31	2.06
	Old:Time(73)	2.68	0.78	3.41	0.001	14.52	2.19
Begging	Young	0.38	0.30	1.27	0.203	1.47	1.35
	Mid-aged	-0.36	0.32	-1.13	0.257	0.70	1.38
	Old	-1.83	0.36	-5.13	< 0.001	0.16	1.43
	Time(25)	0.42	0.30	1.38	0.167	1.52	1.35
	Time(49)	-0.78	0.31	-2.48	0.013	0.46	1.37
	Time(73)	-2.16	0.47	-4.61	< 0.001	0.12	1.60
	Young:Time(25)	0.03	0.38	0.08	0.934	1.03	1.46
	Mid-aged:Time(25)	-0.74	0.48	-1.54	0.123	0.48	1.62
	Old:Time(25)	-0.53	0.50	-1.06	0.291	0.59	1.65
	Young:Time(49)	1.08	0.39	2.76	0.006	2.95	1.48
	Mid-aged:Time(49)	1.14	0.43	2.66	0.008	3.11	1.53
	Old:Time(49)	0.70	0.48	1.47	0.141	2.02	1.61
	Young:Time(73)	2.98	0.59	5.07	< 0.001	19.64	1.80
	Mid-aged:Time(73)	2.12	0.58	3.69	< 0.001	8.35	1.78
	Old:Time(73)	2.03	0.60	3.39	0.001	7.64	1.82
Conditional provisioning	Young	0.30	0.37	0.82	0.410	1.35	1.45
	Mid-aged	-0.43	0.38	-1.13	0.258	0.65	1.46
	Old	-1.43	0.43	-3.33	0.001	0.24	1.54
	Time(25)	0.01	0.39	0.01	0.988	1.01	1.47
	Time(49)	-0.68	0.41	-1.65	0.099	0.51	1.51
	Time(73)	-2.21	0.63	-3.52	< 0.001	0.11	1.87
	Brood size	-0.09	0.05	-1.86	0.063	0.91	1.05
	Carcass mass	0.28	0.08	3.41	0.001	1.32	1.09
	Young:Time(25)	0.25	0.45	0.56	0.578	1.28	1.57
	Mid-aged:Time(25)	-0.44	0.56	-0.79	0.427	0.64	1.75
	Old:Time(25)	-0.32	0.61	-0.52	0.604	0.73	1.84
	Young:Time(49)	0.61	0.48	1.26	0.207	1.84	1.62
	Mid-aged:Time(49)	1.17	0.52	2.26	0.024	3.21	1.68
	Old:Time(49)	0.62	0.59	1.04	0.297	1.85	1.81
	Young:Time(73)	2.25	0.73	3.08	0.002	9.47	2.08
	Mid-aged:Time(73)	2.43	0.73	3.35	0.001	11.4	2.07
	Old:Time(73)	1.97	0.77	2.55	0.011	7.16	2.16
Total care	Young	0.39	0.22	1.76	0.078	1.47	1.25
	Mid-aged	0.73	0.24	3.09	0.002	2.08	1.27
	Old	0.72	0.25	2.92	0.004	2.05	1.28
	Time(25)	-0.02	0.21	-0.07	0.941	0.98	1.24
	Time(49)	0.14	0.22	0.61	0.542	1.14	1.25
	Time(73)	0.19	0.25	0.75	0.453	1.20	1.28
	Brood size	-0.07	0.04	-1.73	0.084	0.94	1.04
	Carcass mass	0.11	0.05	2.19	0.028	1.12	1.05
	Young:Time(25)	-0.11	0.27	-0.42	0.676	0.89	1.31
	Mid-aged:Time(25)	-0.70	0.30	-2.33	0.020	0.50	1.35
	Old:Time(25)	-0.70	0.30	-2.30	0.021	0.50	1.35
	Young:Time(49)	-0.49	0.28	-1.75	0.080	0.61	1.33
	Mid-aged:Time(49)	-0.56	0.30	-1.85	0.065	0.57	1.35
	Old:Time(49)	-0.39	0.30	-1.31	0.192	0.68	1.35
	Young:Time(73)	-0.94	0.40	-2.34	0.019	0.39	1.49
	Mid-aged:Time(73)	-0.88	0.34	-2.58	0.010	0.42	1.41
	Old:Time(73)	-0.66	0.32	-2.05	0.041	0.52	1.38
Larval interactions	Young	0.73	0.65	1.13	0.259	2.08	1.91
	Mid-aged	2.41	0.60	4.00	< 0.001	11.16	1.83
	Old	2.82	0.60	4.71	< 0.001	16.72	1.82
	Time(25)	1.18	0.66	1.77	0.076	3.25	1.94
	Time(49)	1.21	0.66	1.84	0.066	3.37	1.93
	Time(73)	0.81	0.72	1.13	0.260	2.24	2.05
	Young:Time(25)	-1.21	0.79	-1.52	0.128	0.30	2.21
	Mid-aged:Time(25)	-2.78	0.80	-3.49	< 0.001	0.06	2.22
	Old:Time(25)	-2.55	0.75	-3.38	0.001	0.08	2.13
	Young:Time(49)	-1.51	0.81	-1.87	0.062	0.22	2.25
	Mid-aged:Time(49)	-2.22	0.76	-2.92	0.004	0.11	2.14
	Old:Time(49)	-2.73	0.75	-3.65	< 0.001	0.07	2.11
	Young:Time(73)	-1.14	0.90	-1.27	0.203	0.32	2.45
	Mid-aged:Time(73)	-2.47	0.86	-2.87	0.004	0.08	2.36
	Old:Time(73)	-1.78	0.78	-2.28	0.023	0.17	2.18

Appendix B.

Supplementary material for Chapter 4

Analyses of other parental behaviours in Chapter 4

Appendix B:

Analyses of parental behaviours in Chapter 4

In each behaviour observation conducted during the experiment, I categorised the behaviour of the female *Nicrophorus vespilloides* parent at each scan into seven distinct categories: feeding the larvae, interacting with the larvae, guarding the larvae, maintaining the carcass, consuming the carcass, nonparental behaviours, and being away from the carcass altogether. I also recorded whether the female was within a pronotum's length distance away from the larvae, thus being close enough to them to trigger begging (trait hereafter referred to as proximity). Each behaviour was analysed separately to investigate any potential confounding factors in the data. I do not present the data for feeding separately, as it is very close to the data for provisioning: For feeding, I only counted the number of scans when the female was provisioning, whereas counts for provisioning also take into account the number of larvae being provisioned for at any given time. Here I present results of analyses on behaviours that were not presented in the main chapter: indirect care (see below for the definition), consuming the carcass, nonparental behaviours and time spent away, as well as female's proximity to the larvae.

I summarised behaviours that consisted of the female providing care to the off-

Table B.1. Indirect care behaviours exhibited by the female burying beetle *Nicrophorus vespilloides* during the 30 min behaviour observations. For each factor on the treatment level, there is information on parameter estimate (Par), standard error (SE), test statistic (Z value), P value (P), effect size (ES), and the standard error of the effect size (SE(ES)). Analyses were conducted with generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors.

Factor	Par	SE	Z value	*P*	ES	SE(ES)
Young	0.30	0.24	1.26	0.208	1.36	1.27
Mid-aged	0.56	0.26	2.15	0.032	1.75	1.30
Old	0.67	0.27	2.45	0.014	1.95	1.31
Time(25)	-0.36	0.24	-1.52	0.127	0.70	1.27
Time(49)	0.21	0.24	0.85	0.393	1.23	1.27
Time(73)	0.36	0.26	1.38	0.167	1.44	1.30
Brood size	0.11	0.06	1.95	0.052	1.11	1.06
Young:Time(25)	0.15	0.30	0.51	0.610	1.16	1.35
Mid-aged:Time(25)	-0.05	0.33	-0.14	0.887	0.95	1.39
Old:Time(25)	-0.21	0.33	-0.63	0.530	0.81	1.39
Young:Time(49)	-0.73	0.31	-2.39	0.017	0.48	1.36
Mid-aged:Time(49)	-0.66	0.33	-2.04	0.042	0.51	1.39
Old:Time(49)	-0.15	0.32	-0.48	0.633	0.86	1.38
Young:Time(73)	-2.05	0.40	-5.19	< 0.001	0.13	1.49
Mid-aged:Time(73)	-0.92	0.38	-2.44	0.015	0.40	1.46
Old:Time(73)	-0.69	0.34	-2.02	0.043	0.50	1.41

Table B.2. Occurences of the female burying beetles *Nicrophorus vespilloides* consuming the carrion during the 30 min behaviour observations. For each factor on the treatment level, there is information on parameter estimate (Par), standard error (SE), test statistic (Z value), P value (P), effect size (ES), and the standard error of the effect size (SE(ES)). Analyses were conducted with generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors.

Factor	Par	SE	Z value	*P*	ES	SE(ES)
Young	0.26	0.40	0.66	0.511	1.30	1.48
Mid-aged	-0.18	0.42	-0.42	0.674	0.84	1.52
Old	-0.25	0.47	-0.53	0.595	0.78	1.59
Time(25)	0.38	0.34	1.10	0.270	1.46	1.41
Time(49)	0.14	0.38	0.36	0.719	1.15	1.46
Time(73)	-0.68	0.56	-1.22	0.221	0.51	1.75
Young:Time(25)	-0.21	0.42	-0.49	0.621	0.81	1.53
Mid-aged:Time(25)	0.11	0.52	0.21	0.835	1.11	1.69
Old:Time(25)	0.50	0.49	1.02	0.309	1.65	1.63
Young:Time(49)	-0.15	0.46	-0.32	0.752	0.86	1.59
Mid-aged:Time(49)	0.65	0.47	1.38	0.168	1.92	1.61
Old:Time(49)	0.40	0.54	0.74	0.458	1.49	1.71
Young:Time(73)	0.47	0.72	0.65	0.517	1.59	2.06
Mid-aged:Time(73)	1.48	0.64	2.31	0.021	4.39	1.90
Old:Time(73)	0.39	0.76	0.52	0.603	1.48	2.13

spring indirectly (guarding the larvae and maintaining the carcass) into one category (similarly to Mattey and Smiseth, 2015; Walling et al., 2008). This was done as guarding is a very rare behaviour, and it was observed equally rarely in all observations conducted and provided no additional insight into the behaviours exhibited by the females. Non-parental behaviours encompassed a multitude of behaviours such as the female grooming itself, ruminating, walking or hiding under the carcass, all of which were categorized as non-parental during the observations. All traits were treated as counts.

Statistical analyses

All analyses were conducted with R version 3.1.1 (R Core Team, 2013)). The behaviour traits had zero-inflated negative binomial error structures, and as such they were analysed using generalized linear mixed-effects models (R package glmmADMB, Fournier et al., 2012; Skaug et al., 2014). In all models, I assigned experimental treatment (control, young, mid-aged or old), time of observation (1 hour, 25 hours, 49 hours or 73 hours after the start of the experiment), and the interaction between the two, as fixed effects, and block and the identity of the female (to control for pseudoreplication) as random factors. I included the mass of the mouse, and the size of the brood as covariates in the models. After the full models were fitted, the non-significant covariates were dropped in a step-wise simplification based on the maximum likelihood estimates obtained from ANOVAs between nested models, and only terms significant in $P < 0.05$ level were retained

Table B.3. Occurences of the female burying beetles *Nicrophorus vespilloides* exhibiting non-parental behaviours during the 30 min behaviour observations. For each factor on the treatment level, there is information on parameter estimate (Par), standard error (SE), test statistic (Z value), P value (P), effect size (ES), and the standard error of the effect size (SE(ES)). Analyses were conducted with generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors.

Factor	Par	SE	Z value	*P*	ES	SE(ES)
Young	-0.32	0.26	-1.26	0.209	0.72	1.29
Mid-aged	-0.87	0.30	-2.88	0.004	0.42	1.35
Old	-0.89	0.31	-2.84	0.004	0.41	1.37
Time(25)	0.10	0.23	0.46	0.647	1.11	1.26
Time(49)	0.12	0.23	0.51	0.607	1.12	1.26
Time(73)	-0.20	0.26	-0.77	0.441	0.82	1.30
Brood size	0.08	0.04	1.99	0.047	1.08	1.04
Carcass mass	-0.06	0.04	-1.39	0.163	0.94	1.04
Young:Time(25)	0.22	0.30	0.76	0.449	1.25	1.34
Mid-aged:Time(25)	1.16	0.34	3.44	0.001	3.19	1.40
Old:Time(25)	0.89	0.35	2.51	0.012	2.43	1.42
Young:Time(49)	0.39	0.29	1.34	0.179	1.48	1.34
Mid-aged:Time(49)	0.73	0.36	2.02	0.043	2.08	1.44
Old:Time(49)	0.67	0.35	1.89	0.059	1.95	1.42
Young:Time(73)	0.69	0.37	1.85	0.065	1.99	1.45
Mid-aged:Time(73)	1.13	0.38	3.01	0.003	3.10	1.46
Old:Time(73)	1.18	0.37	3.16	0.002	3.27	1.45

in the models. Both the mass of the mouse and brood size were kept in the model for non-parental behaviours. Mass of the mouse was was dropped from the model for indirect care, and both covariates were dropped from the models for consuming, time spent away and time spent within proximity of the larvae.

Results

Indirect care

Overall, the pattern of indirect care was different in the control treatment than in the three experimental treatments (Table B.1). In the young treatment, the amount of indirect care provided declined towards the end of the experiment (similarly to the amount of total care presented in the main body of the thesis). The amount of indirect care provided was more stable in the mid-age treatment, though it was still declining, and the amount of indirect care provided fluctuated in the old treatment (Table B.1). The changes in indirect care behaviours were primarily due to changes in the amount of time the female spent maintaining the carcass, and the general pattern corresponded to that found for total care.

Consuming the carcass

All treatments aside from the mid-aged one followed the pattern of the control treatment for the amount of time spent on consuming the carcass (Table B.2). The pattern of the mid-aged treatment was more variable, and the females consumed more of the carcass at the end of the experiment than the females in the control treatment (Table B.2). Aside from the peak in mid-aged treatment, no deviations from the pattern of the control treatment were detected in the frequency of consuming the carcass.

Non-parental behaviours

The pattern of non-parental behaviours was different in some of the experimental treatments than it was in the control treatment (Table B.3). The females in the control treatment spent less time on non-parental behaviours during the observation conducted 1 h and 73 h into the experiment (Table B.3). The young treatment followed the pattern of the control treatment, but a different pattern was observed for mid-age and old treatments (Table B.3). Both mid-age and old treatments started at lower levels of non-parental behaviours, but the time spent on these behaviours increased in the subsequent observations (Table B.3). Females in the mid-aged and old treatments also spent less time on non-parental behaviours in total (Table B.3), which was to be expected based on the the same

Table S1.4. Occurences of the female burying beetles *Nicrophorus vespilloides* being away from the carcass during the 30 min behaviour observations. For each factor on the treatment level, there is information on parameter estimate (Par), standard error (SE), test statistic (Z value), P value (P), effect size (ES), and the standard error of the effect size (SE(ES)). Analyses were conducted with generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors.

Factor	Par	SE	Z value	*P*	ES	SE(ES)
Young	0.13	0.24	0.54	0.590	1.14	1.28
Mid-aged	-0.02	0.27	-0.09	0.930	0.98	1.31
Old	-0.57	0.29	-2.00	0.046	0.57	1.33
Time(25)	-2.60	0.89	-2.90	0.004	0.07	2.45
Time(49)	0.10	0.40	0.24	0.811	1.10	1.49
Time(73)	0.03	0.26	0.10	0.920	1.03	1.30
Young:Time(25)	2.45	0.94	2.59	0.009	11.53	2.57
Mid-aged:Time(25)	3.15	0.95	3.34	0.001	23.40	2.57
Old:Time(25)	3.11	0.96	3.24	0.001	22.44	2.62
Young:Time(49)	-0.73	0.51	-1.43	0.153	0.48	1.67
Mid-aged:Time(49)	0.38	0.67	0.57	0.567	1.47	1.95
Old:Time(49)	-1.87	1.05	-1.79	0.073	0.15	2.85
Young:Time(73)	0.18	0.32	0.55	0.584	1.19	1.38
Mid-aged:Time(73)	0.42	0.42	1.00	0.317	1.52	1.52
Old:Time(73)	0.48	0.42	1.14	0.253	1.62	1.53

females spending more time on total care (see main text of the thesis).

Time spent away

Females in the old treatment spent less time away overall, and all experimental females spent more time away from the carcass at 25 h after the start of the experiment (Table B.4). After that particular observation time, no deviation from the pattern of the control treatment was detected (Table B.4).

Time spent in the proximity of the larvae

There were no distinct differences in the time spent in the proximity of the larvae between the control treatment and the young and old treatments (table B.5). The females of the mid-aged treatment spent less time in the proximity of the larvae in the observation conducted at 25 h after the start of the experiment, but aside from that there were no differences between the mid-aged treatment and the control treatment (Table B.5).

Table S1.5. Occurences of the female burying beetles *Nicrophorus vespilloides* being within a pronotum length's distance from the larvae (i.e. being in the proximity of the larvae) during the 30 min behaviour observations. For each factor on the treatment level, there is information on parameter estimate (Par), standard error (SE), test statistic (Z value), P value (P), effect size (ES), and the standard error of the effect size (SE(ES)). Analyses were conducted with generalized linear mixed effects models (glmmADMB) with experimental block and the identity of the female assigned as random factors.

Factor	Par	SE	Z value	*P*	ES	SE(ES)
Young	0.20	0.29	0.67	0.502	1.22	1.34
Mid-aged	0.53	0.28	1.89	0.059	1.70	1.33
Old	0.46	0.29	1.57	0.118	1.58	1.34
Time(25)	0.46	0.26	1.75	0.081	1.58	1.30
Time(49)	0.03	0.29	0.11	0.909	1.03	1.34
Time(73)	0.05	0.42	0.11	0.914	1.05	1.53
Young:Time(25)	-0.20	0.34	-0.59	0.553	0.82	1.40
Mid-aged:Time(25)	-1.05	0.53	-1.97	0.049	0.35	1.70
Old:Time(25)	-0.74	0.38	-1.93	0.054	0.48	1.47
Young:Time(49)	0.15	0.37	0.42	0.677	1.17	1.45
Mid-aged:Time(49)	0.15	0.36	0.43	0.670	1.17	1.43
Old:Time(49)	-0.41	0.38	-1.08	0.281	0.67	1.46
Young:Time(73)	-0.13	0.67	-0.19	0.846	0.88	1.96
Mid-aged:Time(73)	-0.02	0.49	-0.04	0.970	0.98	1.62
Old:Time(73)	-0.60	0.50	-1.21	0.227	0.55	1.65

Concluding remarks

All behaviour traits presented here were accounted for in the count for total care provided presented in the main text of the thesis. Indirect care was a part of total care provided, encompassing all behaviours that were not accounted for by the time spent interacting with the larvae (presented in Chapter 4) and feeding. The rest of the behaviours presented here - consuming the carcass, non-parental behaviours, and time spent away - were accounted for as the opposite of total care. Thus, these three behaviours and total care were mutually exclusive. There were no contradictory patterns in the comparisons between the control treatment and the experimental treatments in these three behaviours, suggesting that our measure of total care provided encompassed the main differences in the types of behaviours exhibited by the females. There was also no pattern detectable in the time spent within proximity of the larvae, hence showing that the females were equally likely to be close to the larvae in all treatments, and the differences detected in the behaviours were due to the female behaviour rather than their proximity to the larvae.

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Appendix C.

A paper arising from this thesis

M. I. Mäenpää, C.P. Andrews, D. Collette, D. Leigh and P.T. Smiseth. Burying beetle larvae discriminate between individual parents and between some classes of adults. *Ethology*, 121: 395-402, 2015.

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Burying Beetle Larvae Discriminate Between Individual Parents and Between Some Classes of Adults

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Abstract

Offspring begging can be triggered by a variety of acoustic, visual or chemical cues from the parents. In many birds, nestlings use information derived from these cues to discriminate between individual parents or different classes of adults. Although begging occurs in some insects, we know very little about discrimination between adults by insect larvae. Here, we examine whether begging larvae in the burying beetle *Nicrophorus vespilloides* can discriminate between individual parents or different classes of adults. We found that larvae showed no discrimination between male and female beetles, but that they begged more towards breeding beetles than towards non-breeding ones. These results were robust regardless of whether larvae had been reared in presence or absence of adult beetles, thus suggesting that larval discrimination is based on an innate template that requires no prior exposure to adult beetles. We also found that larvae begged more towards unfamiliar beetles than towards familiar ones, suggesting that they can learn to discriminate between individual parents based on cues about familiarity. We conclude that insect larvae may benefit from discriminating between different classes of adult beetles, as it allows them to lower the costs associated with begging in response to irrelevant environmental cues (costly in terms of wasted effort) and with not begging in response to the presence of caring parents (costly in terms of lost feeding opportunities).

Introduction

Offspring of many birds and mammals, as well as some insects, beg for food from their parents (Kilner & Johnstone 1997). In many birds, begging is triggered by acoustic or visual cues from the parents, such as the feeding calls announcing the parent's arrival in many songbirds (e.g. Leonard & Horn 2001; Madden et al. 2005) or the red patch on the parent's bill in herring gulls *Larus argentatus* (Tinbergen 1948; ten Cate et al. 2009). This phenomenon is thought to reflect selection on offspring to reduce the costs associated with the failure to start begging as the parent arrives at the nest (Budden & Wright 2001; Leonard & Horn 2001), and mistaken responses to irrelevant environmental noises (Leonard et al. 1997). In many birds, nestlings use information derived from these parental cues to discriminate between individual parents or different classes of adults (Lessells et al. 1995). For example, in king penguins *Aptenodytes patagonicus*, parents produce individually distinctive feeding calls, which chicks use to discriminate their parents from other adults in the breeding colony (Aubin & Jouventin 1998). Although begging also occurs in some insects, there is little information on the offspring's ability to discriminate between individual parents or different classes of adults.

The burying beetle *Nicrophorus vespilloides* is an excellent system for studying begging (Smiseth & Moore 2002, 2004a; Smiseth et al. 2003, 2007a,b, 2010). Like all members of its genus, this species breeds on carcasses of small vertebrates (Scott 1998).

Once a suitable carcass has been located, a male and a female normally cooperate by burying it underground, rolling it into a ball and removing fur or feathers (Scott 1998). One or both parents provide care for the larvae by cleaning the carcass of microbial growth, defending the brood against predators and conspecific intruders, and provisioning regurgitated carrion to the larvae (Scott 1998). Larvae beg for food from the parents by raising their heads and touching the parent (Smiseth et al. 2003). As predicted by theoretical models of begging as an honest signal (Godfray 1991), begging reflects larval hunger levels (Smiseth & Moore 2004a), parents adjust their food provisioning in response to begging (Smiseth & Moore 2002), and begging is costly to the larvae (Andrews & Smiseth 2013).

Previous work shows that larval begging in *N. vespilloides* is triggered by chemical cues from the parents and that larvae use these cues to discriminate between breeding and non-breeding females (Smiseth et al. 2010), but not between parents in different stages of breeding (Leigh & Smiseth 2012). There is good evidence that breeding and non-breeding beetles differ with respect to surface chemicals (cuticular hydrocarbons and methyl geranate), and adult females discriminate between their male breeding partner and non-breeding intruders based on differences in their surface chemical profiles (Müller et al. 2003; Steiger et al. 2007; Haberer et al. 2010). There is also evidence that male and female beetles differ with respect to surface chemicals (Steiger et al. 2009; Haberer et al. 2010), and adult beetles learn to recognise individual differences in chemical cues (Steiger et al. 2008). In this study, we examine whether larvae can discriminate between different classes of adults based on their sex and breeding status. We also examine the role of learning by testing whether the larvae's ability to discriminate between specific parental cues requires prior exposure to breeding adults. Finally, we examine whether larvae can learn to recognise individual differences in parental cues.

Methods

Origin and Husbandry of Beetles

The beetles used in the experiments originated from an outbred laboratory population maintained at The University of Edinburgh, UK. The population descended from beetles caught in Corstorphine Hill and Craiglockhart Hill (Edinburgh, UK), and Kennall Vale (Cornwall, UK). All beetles were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil, and kept under constant light at 20°C. Non-breeding beetles were fed small pieces of organic beef twice a week. For breeding, we randomly selected pairs of non-sibling males and females and placed them in a plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (10–20 g; supplied by Livefoods Direct, Sheffield, UK).

General Experimental Procedures

Across all experiments, we adopted the general protocol for recording larval begging in standardised broods comprised of 10 same-aged larvae presented with a standardised stimulus in the form of a dead adult beetle from a specific treatment group (Smiseth & Parker 2008; Smiseth et al. 2010). This protocol provides an experimental procedure for excluding confounding effects due to variation in the size and age-composition of the brood (Smiseth et al. 2003, 2007a,b) or the behaviour of adults (Smiseth et al. 2010). To generate experimental broods, we moved the breeding pair and the carcass to a fresh container 65 h after pairing, thereby leaving the eggs to hatch in the original container. We checked the original container multiple times each day for the presence of newly hatched larvae, which we used to generate experimental broods that were always comprised of mixed-maternity larvae (for further details on the protocol and rationale for use of mixed-maternity broods, see Smiseth et al. 2010). We allocated experimental broods randomly to foster parents, only using beetles whose own eggs had started hatching to avoid filial cannibalism (Bartlett 1987).

We recorded larval begging 24 h (±20 min) after the experimental broods had been generated to coincide with the stage in larval development when begging peaks (Smiseth et al. 2003). Thirty minutes before we started recording larval begging, we removed the adult beetle to be used as a stimulus (see Experimental Design for further details). We then killed the beetle by placing it in a −20°C freezer for 20 min and left it to thaw for another 5 min before pinning it within a small plastic container (12 × 8 cm × 2 cm) lined with a moist paper towel (see Smiseth et al. 2010). The beetle was pinned in a position mimicking that of a parent regurgitating food. Once the beetle had been pinned, we removed the larvae and placed them next to the pinned beetle. We waited 5 min before starting the observations to give the larvae time to settle. We recorded larval begging using instantaneous recording every 1 min during a 30-min

observation period according to established protocols (Smiseth & Moore 2002). The average time spent begging by each larva in the brood, B, was calculated as $B = (b/l)/30$, where b is the total number of begging events during the 30-min observation period and l is the mean number of larvae near the adult during each scan (i.e. within 0.8 cm diameter from the pin). The total sample size across all three experiments amounted to 132 broods.

Experimental Design

Experiment 1: In this experiment, we tested whether larvae that had been reared in the presence of both a male and a female beetle discriminated between the two sexes. We left both a male and a female beetle with the brood until we conducted the behavioural observations to ensure that the larvae had been exposed to the chemical profiles of both parents. Males often desert the brood during the first 24 h after hatching (Smiseth et al. 2005), and it is currently unclear whether the chemical profile of deserting males resembles that of caring males. We therefore recorded all instances of male desertion, by checking twice whether the male (and female) was present on the carcass or the surrounding crypt before conducting the behavioural observations. We did the first check 30 min before the removal of the target parent and the second one immediately prior to it. If the male was absent from the carcass during both checks, we scored him as having deserted the brood. In summary, in this experiment, the larvae were presented with a standardised stimulus in the form of a dead adult from one of the following three categories: a caring female (n = 10), a caring male (n = 10) or a deserting male (n = 10). We presented all larvae with one of the adults that previously had provided care for them (hereafter referred to as a caring parent) to exclude any potential confounding effects due to the familiarity of the adult.

Experiment 2: This experiment was designed to test whether larvae that had been reared by a single male or female beetle discriminated between adults based on their breeding status or familiarity. We also tested whether any discrimination based on cues about the breeding status and familiarity was conditional on the adult's sex. We always removed one member of the breeding pair at the time we generated the experimental broods, thereby leaving the remaining beetle to provide care on its own during the first 24 h after hatching. We then presented the larvae with an adult beetle of the same sex as the beetle that previously had cared for them. We used a single-parent design because, when both parents care jointly, females tend to spend more time interacting with the larvae than males. In contrast, there is no difference in the amount of time that single males and single females spend interacting with the larvae (Smiseth et al. 2005). Thus, this design allowed us to exclude any potential confounding effects that may arise in Experiment 1 due to females interacting more with the larvae.

Previous work shows that larvae respond to cues about the breeding status of females (Smiseth et al. 2010), but there is no information on larval responses to cues from males. Thus, we tested whether larval discrimination between breeding and non-breeding adults was conditional on the adult's sex. To this end, we presented the larvae with either a breeding or non-breeding adult. There were no instances of desertion in this experiment, and all breeding beetles were caring for the larvae. Given that non-breeding adults inevitably will be unfamiliar to the larvae, we presented the larvae with an unfamiliar breeding adult as a control. Finally, this experiment also allowed us to examine whether larvae can learn to recognise individual differences in chemical cues, as previously reported for adults (Steiger et al. 2008). If so, we expected larvae to discriminate among familiar and unfamiliar breeding adults. Thus, we presented some larvae with the adult that had previously provided care for them (hereafter referred to as a familiar breeding parent) and some larvae with an adult that had previously cared for a different brood (hereafter referred to as an unfamiliar breeding parent). In summary, the larvae used in this experiment were presented with a dead male or female beetle from one of the following treatments: a familiar breeding beetle (n = 11 and n = 10 for females and males, respectively), an unfamiliar breeding beetle (n = 10 and n = 10 for females and males, respectively) or an unfamiliar non-breeding beetle (n = 9 and n = 10 for females and males, respectively).

Experiment 3: In this experiment, we tested whether the larvae's ability to discriminate between adults based on cues about breeding status and sex required that the larvae had previously interacted with adult beetles. In *N. vespilloides*, larvae obtain some food by self-feeding, and they survive well without post-hatching parental care (Eggert et al. 1998; Smiseth et al. 2003). Thus, we reared larvae in isolation from caring parents during the first 24 h after hatching by removing both parents at the time we generated the experimental broods. We then examined whether these larvae discriminated between adults based on breeding status and sex by presenting

them with a dead male or female from one of the following two treatments: a breeding beetle (n = 11 and n = 8 for females and males, respectively) or a non-breeding beetle (n = 10 and n = 13 for females and males, respectively).

Statistical Analysis

All analyses were conducted using R version 3.0.2 (R Core Team 2013). We used a logit transformation with 0.001 as the constant ε to reduce heteroscedasticity and normalise the error structure of the proportional data on larval begging (Warton & Hui 2011). The data were collected in multiple experimental blocks conducted at different times over 3 years. We used linear mixed-effects models (lme, package nlme) to analyse data on all experiments, with block as a random effect. For Experiment 1, we used treatment (caring female, caring male, deserting male) as a fixed factor. This analysis allowed us to test for a difference in larval begging towards male and female beetles and towards caring and deserting males. For Experiment 2, we used treatment (the breeding status and familiarity of the adults, that is familiar breeding beetles, unfamiliar breeding beetles, unfamiliar non-breeding beetles) and sex of the adult (females, males) as fixed factors. This analysis allowed us to test for a difference in larval begging towards male and female beetles, towards familiar and unfamiliar breeding adults and towards unfamiliar breeding and non-breeding adults. For Experiment 3, we used breeding status (breeding beetles, non-breeding beetles) and sex of the adult (females, males) as fixed factors. This analysis allowed us to test for a difference in larval begging towards breeding and non-breeding adults. Contrasts comparing the different levels of significant main effects were computed to identify differences between groups for treatments that had more than two levels.

Results

Experiment 1: There were no significant differences in the amount of time the larvae spent begging towards caring females, caring males or deserting males (lme, $F_{2,25} = 0.193$, $p = 0.826$). Thus, larvae that had been reared by both parents did not discriminate between caring parents based on their sex or between caring males and males that had deserted the brood (Fig. 1).

Experiment 2: Larvae spent a similar amount of time begging towards females and males when they had been reared by either a single female or a single male beetle (lme, $F_{1,52} = 0.114$, $p = 0.737$). Thus, as in Experiment 1, larvae did not discriminate between adults based on their sex (Fig. 2). However, there was a highly significant effect of treatment (i.e. the breeding status and familiarity of adults) on the amount of time that the larvae spent begging (lme, $F_{2,52} = 42.6$, $p < 0.001$). Post hoc contrasts show that the larvae spent significantly more time begging towards unfamiliar breeding beetles than towards familiar ones ($z_{54} = 6.65$, $p < 0.001$, Fig. 2). In addition, larvae spent significantly more time begging towards unfamiliar breeding beetles than towards unfamiliar non-breeding beetles ($z_{54} = 6.08$, $p < 0.001$, Fig. 2). There was no significant effect of the interaction between treatment and sex on larval begging (lme, $F_{2,52} = 2.21$, $p = 0.137$).

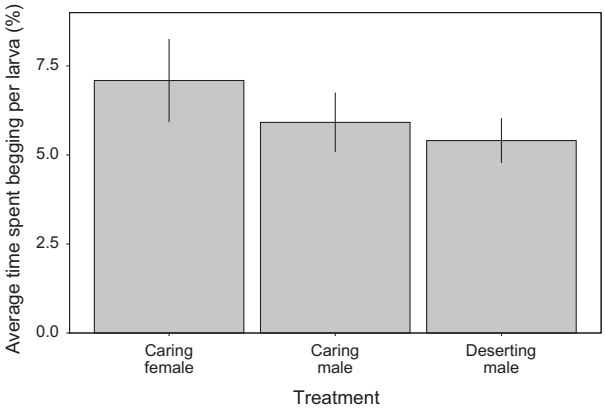

Fig. 1: Mean (±1 SE) time spent begging by individual larvae (%) towards a dead parent. In this experiment, both parents had reared the larvae. There were no significant differences in the amount of time spent begging towards different classes of adults ($p > 0.05$).

Experiment 3: There was no significant difference in the amount of time spent begging towards females and males when larvae had been reared in isolation from any contact with adult beetles (lme, $F_{1,36} = 1.58$, $p = 0.217$). Thus, as in the previous two experiments, larvae did not discriminate between adults based on cues about their sex (Fig. 3). As in Experiment 2, there was a significant difference in the amount of time spent begging towards breeding and non-breeding beetles (lme, $F_{1,36} = 4.17$, $p = 0.049$). Although visual inspection of the data seems to indicate a differential response to the treatments between males and females (Fig. 3), there was no significant effect of interaction term between treatment and sex (lme, $F_{1,36} = 2.85$, $p = 0.100$).

Discussion

Here, we provide evidence that burying beetle larvae discriminate between certain classes of adults and

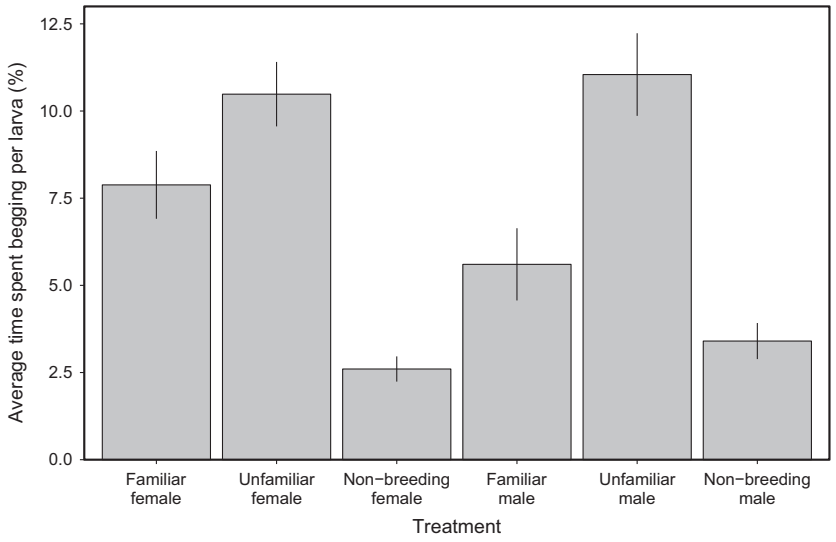

Fig. 2: Mean (±1 SE) time spent begging by individual larvae (%) towards a dead parent. In this experiment, either a single male or a single female had reared the larvae. There were no significant differences in the amount of time the larvae spent begging towards a male or a female beetle ($p > 0.05$), but the differences across all treatment types (familiar, unfamiliar and non-breeding) were statistically significant ($p < 0.05$).

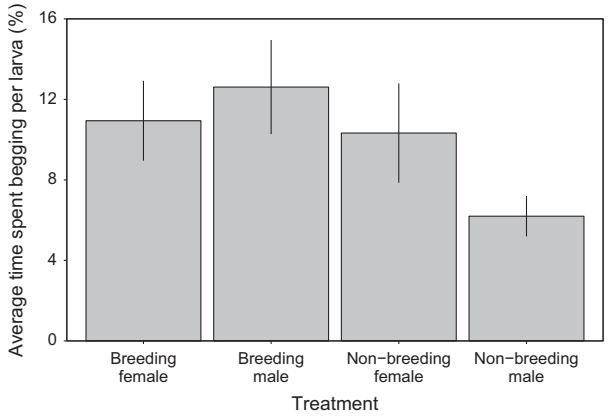

Fig. 3: Mean (±1 SE) time spent begging by individual larvae (%) towards a dead parent. In this experiment, the larvae had been reared in isolation from caring parents. There were no significant differences in the amount of time spent begging towards a male or a female beetle ($p > 0.05$), but the differences in begging towards breeding and non-breeding adult beetles were statistically significant ($p < 0.05$).

between individual parents. We found that larvae did not discriminate between male and female beetles regardless of whether larvae had previously interacted with male and female beetles. There are three potential explanations for why larvae did not discriminate between males and females: (1) there are no cues available to the larvae about the adults' sex, (2) there are such cues but larvae cannot detect them, and (3) larvae can detect the cues but it is beneficial to ignore them. Previous work allows us to exclude the first explanation as it shows that males and females have different surface chemicals (Steiger et al. 2009; Haberer et al. 2010). It is harder to differentiate between the remaining two explanations. There is good evidence that adult females can detect cues about sex given that they are more aggressive towards dead females than towards dead males (Steiger et al. 2009). However, this does not necessarily mean that larvae can detect the same cues, as they may not have the same sensory and cognitive mechanisms for detecting and processing chemical cues as adults. However, even if larvae could detect chemical cues about the adult's sex, it may be detrimental to respond to them. The reason for this is that, even though females spend more time provisioning food to the larvae overall (Eggert et al. 1998; Smiseth & Moore 2002), males and females are equally likely to provision the larvae when in close proximity to them (Smiseth & Moore 2004b). Thus, any reduction in begging towards males is likely to incur a cost in terms of lost feeding opportunities. Based on available evidence, we propose that it would be beneficial for larvae to ignore cues about the parents' sex even if they could detect such cues. Our finding on burying beetles is similar to results from a study on birds, showing that nestlings of European bee-eaters *Merops apiaster* do not discriminate between male and female parents (Lessells et al. 1995).

Larvae begged more towards breeding beetles than towards non-breeding ones regardless of whether they had been reared in the presence or absence of

adult beetles. This is consistent with a previous study showing that larvae spend more time begging towards breeding females than towards non-breeding ones (Smiseth et al. 2010). Our results extend on this work by showing that larval discrimination between adults based on breeding status is independent of the adult's sex and not conditional on prior experiences with adult beetles. There is good evidence that breeding and non-breeding beetles differ with respect to surface chemicals, and adult females are known to use information from these cues to discriminate between their breeding partner and non-breeding intruders (Müller et al. 2003; Steiger et al. 2007; Haberer et al. 2010). It may be beneficial for larvae to respond to chemical cues about the breeding status of adults, because it would provide them with a mechanism with which larvae can reduce the costs associated with discriminating between the parent and irrelevant environmental cues in underground darkness (Smiseth et al. 2010).

The finding that larvae discriminated between breeding and non-breeding adults even when they had been reared in isolation from adult beetles suggests that larval discrimination between parental cues is based on an innate template that requires no prior exposure to adult beetles. Nevertheless, visual inspection of the data suggests that this effect was pronounced when larvae were presented with males but not when larvae were presented with females. Thus, larval discrimination appears to be weaker and less consistent when the larvae were reared in isolation from adults (Experiment 3, Fig 3) than when the larvae were reared by a single parent (Experiment 2, Fig 2). We argue that it would be premature to rule out the possibility that learning might play a role in moderating the larvae's responses towards chemical cues towards parents. Indeed, previous work shows that larvae adjust their begging behaviour in response to the number of competing larvae in brood (Smiseth et al. 2007a) and their own competitive rank relative to that of their siblings (Smiseth et al. 2007b), suggesting that larvae moderate their begging behaviour to the competitive environment in which they find themselves possibly through learning. Furthermore, studies on birds provide good evidence that learning plays an important role in moderating the nestling's begging behaviour (Kedar et al. 2000; Lotem & Biran-Yoeli 2013). Thus, there is a need for further experiments that address the potential role of learning as a mechanism for moderating larval begging behaviour.

Finally, we found that larvae begged more towards unfamiliar beetles than towards familiar ones, suggesting that they can discriminate between individual adult beetles. Our results derive from an experimental design that excluded confounding factors that otherwise covary with familiarity. In natural broods, familiarity covaries with kinship because the larvae tend to be reared by their biological parents (Müller & Eggert 1989). We can exclude effects of kinship on our results because all experimental broods in this study were reared by foster parents. In natural broods, familiarity also covaries with breeding status because any familiar beetle inevitably will be a breeding adult, while any unfamiliar beetle is likely to be a non-breeding intruder (Bartlett 1987; Müller & Eggert 1990). We can also exclude effects due to breeding status because the familiar and unfamiliar adults in our experimental design always had the same breeding status. Surprisingly, we found that larvae spent more time begging towards unfamiliar adults than towards familiar adults, a pattern that was consistent across both sexes. This finding contrasts with a recent study on jackdaws *Corvus monedula*, showing that older nestlings do not discriminate between the calls of their parents and other conspecifics, although they discriminated between conspecific calls and the calls of other corvid species (Zandberg et al. 2014). It is difficult to come up with an adaptive explanation for why larvae should beg more towards unfamiliar beetles. Potentially, this unexpected finding may reflect the outcome of the underlying sensory or cognitive mechanism that controls begging, which caused the larvae to respond in a non-adaptive way in a novel experimental setting (Fawcett et al. 2013). Larvae do not normally encounter unfamiliar non-breeding adults, and if they are neophilic, they may increase their begging when presented with a novel stimulus. Currently, it is unknown whether burying beetle larvae are neophilic, and further work is needed to establish whether they are neophilic or not.

Our results support the suggestion that larval discrimination plays an important role as a mechanism for reducing the costs of begging. Previous work suggests that nestling birds are under selection to reduce the costs associated with the failure to start begging as the parent arrives at the nest (Budden & Wright 2001; Leonard & Horn 2001), and mistaken responses to noise in the environment (Leonard et al. 1997). Our results suggest that burying beetle larvae are under similar selection pressures and that larval discrimination between adults serves as an adaptive mechanism that allows the larvae to lower the costs associated with begging towards irrelevant environmental cues (which is costly in terms of wasted effort) and with not begging in response to the presence of caring parents (which is costly in terms of lost feeding opportunities).

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